2nd Joint European Congress of the ESVP, ESTP and ECVP

32nd Meeting of the European Society of Veterinary Pathology
12th Meeting of the European Society of Toxicologic Pathology
25th Meeting of the European College of Veterinary Pathology

27th – 30th August 2014,
Berlin, Germany

CUTTING EDGE PATHOLOGY

Programme and Abstract Book

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Welcome to Berlin!

Dear fellow pathologists, guests and friends,

We are delighted to welcome you to Berlin, a vibrant metropolis full of history, contemporary arts and lifestyle. Our city is a true melting pot of cultures and nationalities with much room for lively debate. Together with our local team, we will do our very best to provide you with a convenient and relaxing yet professional Congress environment that will allow you to pick up latest scientific trends, keep up with professional developments and be inspired for your own future activities at home. It was our particular goal to shape a laid-back atmosphere in which you can enjoy seeing old friends, meeting new colleagues and making new friends. We look forward to seeing you at the Welcome Reception in the foyer of the Estrel Congress venue as well as at the Congress Dinner in a traditional German brewery in the center of the City, "Berlin Mitte".

The prime objective of the triple "Cutting Edge Pathology" meetings is to bring together the closely related disciplines and specialities to foster professional interactions and enable us to learn from each other. Overall the Congress will offer you a state of the art scientific programme with some of the highlights summarised on page 4–5. A meeting that brings together so many colleagues from a wide spectrum of disciplines and from all over the world will also facilitate a more effective display of their latest technological developments by our industry partners and sponsors. Last but not least, we are almost certain that the wide spectrum of potential employers and employees at the Congress will set the stage for many of you to develop your careers as pathologists, whether you are new trainees or more experienced colleagues, and to network with experts who will enhance your companies, laboratories or academic institutes. We are confident that this formula will ensure continuing success for future triple meetings that will be symbolised by the new brand, Cutting Edge Pathology: the design that was first used for the Uppsala meeting in 2011 and will be used for all future triple meetings.

Berlin was home to Rudolf Virchow, one of the historic giants of pathology who shaped much of our modern understanding of disease. Among several other sightseeing tours and extramural attractions that we organised for you, we cordially invite you to experience the Rudolf Virchow´s personal collection of pathology specimens which is the only one of its kind in the world. For those of you who prefer nightlife or shopping, Berlin is waiting for you.

Please make sure to drink in the triple meeting spirit during the entire Congress: Attend talks and workshops and view posters from all fields of the profession on display! We sincerely hope that the venue and Congress structure will provide you with an ambience that makes your trip to Berlin successful, inspiring, joyful and an experience to remember.

Sincerely,

Your local organisers

Dr. Anna-Lena Frisk
Senior Research Pathologist
Bayer Pharma AG, Berlin

Prof. Dr. Achim D. Gruber, Ph.D.
Chair, Dept. of Veterinary Pathology
Freie Universität Berlin

Local organisers, on behalf of the ESVP, ESTP and ECVP

Local Organising Team

Junior Pathologists at the Institute of Veterinary Pathology, Freie Universität Berlin (from left to right): Lydia König, Hannah Pischon, Nancy Erickson, Anja Ostrowski, Angele Breithaupt, Kristina Dietert, Stephanie Plog, Aleksandra Zuraw, Stefanie Binder, Moritz Radbruch

Olivia Kershaw

Conference Web Page Manager

Pia Schröder

Local organisers assistance

Welcome from the Presidents of the Societies

Frédéric Schorsch, ESTP Chairman

Carl Hård af Segerstad, ESVP President

Sean Callanan, ECVP President

Dear Colleagues

It is a great pleasure to welcome you to our second Joint European Congress of the ESVP, ESTP and ECVP*. The gathering of both our European Societies and College in the historic city of Berlin will ensure a wonderful opportunity to advance our common knowledge and to develop new ideas and novel collaborations. It also offers us the chance to meet with old friends and make new ones.

After our first joint meeting in Uppsala in 2011, your executives decided to renew such a common experience every three years. Most of us have benefited from similar fundamental training experiences in veterinary pathology and then applied our knowledge of comparative medicine in different but interlinked areas. We are therefore delighted to offer you again a Congress where we now can share our different experiences, united by the common bond of pathology and the assurance of the highest international standards.

This conference, aptly named "Cutting Edge Pathology" strives to bring you the most up-to-date advances within pathology. We extend a warm welcome to our plenary speakers and as usual, look forward to wonderful, thought-provoking presentations in areas of toxicopathology of the endocrine and endocrine regulated organs, of nanotoxicology, of regenerative medicine and cancer. Distilling what is new and relevant within these topics will leave us with additional knowledge and guide our thoughts in novel directions.

We must first extend our grateful thanks to our hosts, the local organising committee under the stewardship of Professor Achim Gruber and Dr Anna-Lena Frisk. They have worked tirelessly, with the scientific committees to propose a varied programme of high quality to ensure record attendance and record abstract submissions.

The severe economic challenges and the call for better health and safety from the society continue to impact us worldwide. Our profession within industry, academia and veterinary diagnostic practice do not escape such realities. As a community we continue to strive forward and hold our rightful role in diagnostics, in basic and translational research, in human/animal/ecosystem safety and in toxicological assessments of novel drugs and active materials. We have to adapt our practices to an evolving environment and to new technologies. But morphological evaluations continue to be the corner stone to cutting edge medical imaging, molecular pathology, computational analyses on digital images or big data analysis. These changes call on our 3 organisations to better collaborate. Our joint conference marks a valuable starting point. Over the next few days we will see several examples of such activities within the high quality of the selected oral and poster presentations. We will hear of new diseases, new roles for genes and proteins, new pathogenic mechanisms and new therapeutic strategies. We will also learn about up-to-date International Harmonization of Nomenclature and Diagnostic Criteria which nourishes our daily work.

For those of you just joining us for the first time and those embarking in the discipline of pathology, we give you a warm welcome. Use this opportunity to introduce yourself to as many of your new colleagues as possible as these will be your friends, colleagues and collaborators throughout your career. A critical component of these conferences is also networking at all levels and we can think of a no more beautiful place than Berlin in August.

*It also corresponds to the 32nd annual ESVP Congress, the 25th meeting of the ECVP and the 12th ESTP Congress!

Sincerely

Frédéric Schorsch, ESTP Chairman

Carl Hård af Segerstad, ESVP President

Sean Callanan, ECVP President

Congress Highlights

Scientific Highlights of the Congress

Key Note Lectures

Thomas J. Rosol:	Lessons in Endocrine Pathology from History, Art, and the Microscope
Jens P. Teifke:	Animal Disease Control: Do We Still Need Pathologists?
Ann Hubbs:	Understanding Nanotechnology: An Emerging Challenge for the Toxicologic Pathologist
Wolfgang Baumgärtner:	**The Journal of Comparative Pathology Plenary Award Lecture:** Pathogenesis of Canine Spinal Cord Injury and Development of Cell-Based Treatment
Sven Rottenberg:	Predicting Disease Outcome and Therapy Response in Animal Cancer: Morphology, Immunohistochemical Markers or Molecular Signatures – What to Choose?

ESTP Highlights

Main Topic: Toxicopathology of the Endocrine and Endocrine Regulated Organs with invited speakers

BSTP Chirukandath Gopinath Lecture – Dianne Creasy: Endocrine Regulation and Endocrine Mediated Disturbances in the Adult Male Reproductive System

Nanopathology Session

INHAND Interactive Slide Session *

*__INHAND__ (International Harmonization of Nomenclature and Diagnostic Criteria for Lesions in Rats and Mice) update on endocrine, male and female reproductive tract proliferative and non-proliferative lesions will be presented and discussed.

Pre Meeting- Workshop

Karin Jochims and Barry Drees: Scientific Writing for Toxicopathologists (morning)

Congress Highlights

ESVP / ECVP Highlights

Invited Talks

Sandra Scholes and The European Pathosurveillance Network (EPSN): Pathosurveillance: Past, Present and Future

Michael Day:	Top Tips for Publishing a Research Paper in the Journal of Comparative Pathology
Jeff Caswell and Andrea Gröne:	Secrets of the Journal Revealed: An Analysis of Editorial Decisions from the Editors of Veterinary Pathology
Laura Bongiovanni:	The International Society of Veterinary Dermatopathology (ISVD): Objective, Goals and Future Prospects

Workshops

Robert Klopfleisch:	Lesion Scoring and Digital Image Analysis
Jens P. Teifke:	The Swine Fevers and their Differential Diagnoses
Sandra Scholes / EPSN:	Nomenclature of Respiratory Lesions- does it matter?
Sandra Scholes / EPSN:	New and Re-Emerging and Unusual Disease Presentations in Livestock
Robert Klopfleisch:	Genomics and Proteomics in Veterinary Pathology

Specific Workshops for Trainees

ECVP Exam Committee, Kuno Würsch and Koen Chiers: Tips and Tricks for Exam Preparations

Michael Day: Coaching in Writing and Publishing a Research Paper in the Journal of Comparative Pathology

Industry Exhibition

An **exhibition quiz** is waiting for you: The documents needed for your participation will be handed out to you at the Congress counter. The winner will be announced and a prize awarded on Saturday during the coffee break.

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Wednesday August 27

	ESTP Paris	ECVP/ESVP AB	ESTP C	POSTER HALL
7:00				
7:15				
7:30		Registration Foyer Estrel Hall		
7:45				
8:00				
8:15				
8:30			Workshop: Scientific Writing for Pathologists **Barry Drees:** General Aspects of Scientific Writing for Regulatory Dossiers	
8:45				
9:00			**Karin Jochims:** General Aspects of Communicating Pathology Data	
9:15				
9:30			**Barry Drees:** How to Present Large Data Sets in Regulatory Dossiers	
9:45				
10:00			**Karin Jochims:** Examples of Large Data Sets in the Regulatory Context	
10:15				
10:30			Coffee break	
10:45				
11:00			**Barry Drees:** How to Communicate Terminology in Regulatory Dossiers	
11:15				
11:30			**Karin Jochims:** Examples of Pathology Terminology Presented in Regulatory Dossiers	
11:45				
12:00			**Barry Drees:** How to Integrate Interdisciplinary Data in Regulatory Dossiers	
12:15				
12:30			**Karin Jochims:** Examples of Integrative Pathology Data	
12:45				
13:00	Registration Foyer Estrel Hall	Registration Foyer Estrel Hall	Lunch	Mounting of ESTP Posters
13:15				
13:30				
13:45	**Wolfgang Kaufmann:** INHAND-Introduction			
14:00	**INHAND 01 – Interactive Slide Session** **Dianne Creasy / Eveline de Rijk:** Proliferative and Non-proliferative Lesions of the Rat and Mouse Male Reproductive System			
14:15				
14:30				
14:45				
15:00	**INHAND 02 – Interactive Slide Session** Ute Bach / Justin Vidal: Female Reproductive System			
15:15				
15:30				
15:45				
16:00	Coffee Break			
16:15	**INHAND 03(1-3) – Interactive Slide Sessions** Annamaria Brändli-Baiocco: Non-Proliferative and Proliferative Lesions of the Adrenal Gland in Rodents **Susanne Rittinghausen:** Proliferative Lesions of the Pituitary and Pineal Gland in Rodents **Emmanuelle Balme:** Proliferative Lesions of the Thyroid and Parathyroid Gland in Rodents			Mounting of ECVP Posters, Block I
16:30				
16:45				
17:00				
17:15		Registration Foyer Estrel Hall		Poster Viewing
17:30				
17:45				
18:00				
18:15				
18:30		Welcome Reception Foyer Estrel Hall		
18:45				
19:00				ESTP Posters
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19:30				
19:45				
20:00				
20:15				
20:30				
20:45				
21:00				

Programme Overview

Thursday August 28

	ESTP Paris	ECVP/ESVP AB	ECVP/ESVP C	POSTER HALL	SEMINARS Lyon
7:00					
7:15		Registration Foyer Estrel Hall		Poster Viewing	
7:30					
7:45				ESTP Posters	
8:00					
8:15					
8:30		Welcome with Selected Speakers and Guests			
8:45		Plenary Lecture Tom Rosol: Lessons in Endocrine Pathology from History, Art, and the Microscope			
9:00					
9:15					
9:30					
9:45		Plenary Lecture Jens Peter Teifke: Animal Disease Control – Do We Still Need Pathologists?			
10:00					ESVP / ECVP Scientific Committee Meeting
10:15					
10:30	Coffee Break			ESTP Posters & Authors ECVP Poster Walks: 1a, Oncology I 1b, Degenerative Lesions	
10:45					
11:00	Duncan Ferguson: Primer of Comparative Thyroidology: Relevance to Animal Models of Thyroid Disruption	Sandra Scholes: European Pathosurveillance Network (EPSN): Pathosurveillance – Past, Present and Future			
11:15					
11:30					
11:45					
12:00	Sibylle Gröters: Endocrine Disruptor Testing Mixtures of Endocrine Disruptors *in vivo* at Human-Relevant Exposure Levels	With Subject Related Oral Presentations	Oral Presentations, Session 1: Reproduction		Robert Klopfleisch: Lesion Scoring and Digital Image Analysis
12:15					
12:30					
12:45	Lunch and Industrial Exhibition	Leica Microsystems: Peer Review through Digital Pathology: The Future is Now?	Lunch and Industrial Exhibition		
13:00					
13:15		Lunch and Industrial Exhibition		ESTP Posters with Authors ECVP Poster Walks: 2a, Oncology II 2b, Fundamental Research	
13:30					
13:45					
14:00					
14:15	Dianne Creasy *(The BSTP Chirukandath Gopinath Lecture):* Endocrine Regulation and Endocrine Mediated Disturbances in the Adult Male Reproductive System	Oral Presentations, Session 2a: Oncology I: Mammary Cancer	Oral Presentations, Session 2b: Spontaneous and Experimental Neuropathology		EPSN 1: The Swine Fevers and their Differential Diagnoses (Workshop led by J.Teifke)
14:30					
14:45					EPSN 2: Nomenclature of Respiratory Lesions - does it matter? Discussion Topic
15:00					
15:15	Niels Skakkebaek: Endocrine Disruptors, Male Reproductive Disorders and Low Fertility Rates				EPSN 3: Short Oral Presentations on New / Re-emerging and Unusual Disease Presentations in Livestock
15:30					
15:45					
16:00	Coffee Break			ESTP Posters & Authors Poster Viewing	
16:15					
16:30	Karen Mandrup: Effects on Rat Reproductive System after Developmental Exposure to Mix-tures of Endocrine Disrupters Compounds	Oral Presentations, Session 3a: Oncology II: Spontaneous and Experimental Oncology	Oral Presentations, Session 3b: Toxicology		ECVP Exam Committee, Kuno Würsch and Koen Chiers: Tips and Tricks for Exam Preparations
16:45					
17:00	Hans Harleman: Evaluation of the Mammary Gland in Safety Studies			ECVP Poster Walks: 3a, Oncology III & Immunology 3b, Varia I	
17:15					
17:30					
17:45					
18:00	ESTP Annual Meeting				
18:15					
18:30				Poster Viewing	
18:45					
19:00				Removal of Posters Block I	
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21:00					

Programme Overview

Friday August 29

	ESTP			POSTER HALL	SEMINARS
	Paris	AB	C		Lyon
7:00					
7:15		Registration Foyer Estrel Hall		Mounting of ECVP Posters, Block II	
7:30					
7:45				Poster Viewing	
8:00					
8:15		Michael Day: Top Tips for Publishing a Research Paper in the Journal of Comparative Pathology	Oral Presentations, Session 4: Emerging and Other Infectious Diseases of Current Interest		
8:30	Tom Rosol: Pituitary Gland				
8:45					
9:00		Jeff Caswell / Andrea Gröne: Secrets of the Journal Revealed: An Analysis of Editorial Decisions from the Editors of Veterinary Pathology			
9:15	Tom Rosol: Adrenal Gland				
9:30					
9:45				ECVP Poster Walks: 4a, Emerging and other Infectious Diseases I 4b, Congenital Diseases & Zoo and Wildlife	
10:00	Coffee Break				
10:15					SFTP Poster Award Committee Meeting
10:30	Justin Vidal: Female Reproductive System Part I: Endocrinology and Evaluation of the Female Reproductive System in General Toxicology Studies	Laura Bongiovanni, Sonya Bettenay, Verena Affolter, Chiara Brachelente, Paola Roccobianca: Mystery Slides in Veterinary Dermatopathology – Interactive Slide Session	Oral Presentations, Session 5: Zoo and Wild Life Diseases		
10:45					
11:00					
11:15	Justin Vidal: Female Reproductive System Part II: Mechanisms and Patterns of Toxicity in the Female Reproductive System				
11:30				ECVP Poster Walks: 5a, Emerging and other Infectious Diseases II 5b, Reptiles, Amphibians and Fish I	
11:45					
12:00			Lunch		
12:15		ECVP Annual Meeting (12:20 – 1:20)			
12:30					
12:45					
13:00				ESTP Posters	
13:15					
13:30					
13:45	Maria Cecilia Rey Moreno: Placenta of the Rat – Histology and Pathology	Oral Presentations, Session 6a: Host Pathogen Interactions	Oral Presentations, Session 6b: Outstanding Case Reports		Michael Day: Coaching in Writing and Publishing a Research Paper in the Journal of Comparative Pathology
14:00					
14:15					
14:30	Eberhard Buse: The Non-Human Primate Placenta			ECVP Poster Walks: 6a, Reptiles, Amphibians and Fish II & Emerging and other Infectious Diseases III 6b, Toxicology & Varia II	
14:45					
15:00					
15:15	SFTP / ESTP / IFSTP Awards	Coffee Break			
15:30	Coffee Break				
15:45		Plenary Lecture Ann Hubbs: Understanding Nanotechnology: An Emerging Challenge for the Toxicologic Pathologist			
16:00					
16:15					
16:30					
16:45		Christian Plank: Stabilized Non-Immunogenic Messenger RNA for Transcript Therapy			Robert Klopfleisch: Genomics and Proteomics in Veterinary Pathology
17:00					
17:15		Oral Presentations, Session 7a: Nanotoxicopathology	Oral Presentations, Session 7b: Methodological Developments		
17:30					
17:45		ESVP Annual Meeting		Poster Viewing	
18:00					
18:15				ESTP Poster	
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19:45	Congress Dinner / ECVP Diploma Ceremony				
20:00					
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20:30					
20:45					
21:00					

Programme Overview

Saturday August 30

	ESTP	ECVP/ESVP	ECVP/ESVP	POSTER HALL	SEMINARS
	Paris	AB	C		Lyon
7:00					
7:15					
7:30					
7:45					
8:00					
8:15					
8:30		Journal of Comparative Pathology Plenary Lecture: Wolfgang Baumgärtner: Pathogenesis of Canine Spinal Cord Injury and Development of Cell-Based Treatment Options			
8:45					
9:00					
9:15		Case Presentations – Interactive Slide Session			
9:30					
9:45					
10:00					
10:15					
10:30		Coffee Break		Poster Viewing	
10:45					
11:00					
11:15		Case Presentations – Interactive Slide Session			
11:30					
11:45					
12:00					
12:15		Plenary Lecture: Sven Rottenberg: Predicting Disease Outcome and Therapy Response in Cancer: Morphology, Immunohistochemical Markers ...			
12:30					
12:45					
13:00		Closing Ceremony: Including Awards Ceremony for Best Oral and Poster Presentations		Poster Viewing	
13:15					
13:30				Removal of Posters Block II	
13:45					
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14:30	Post Venue Tour				
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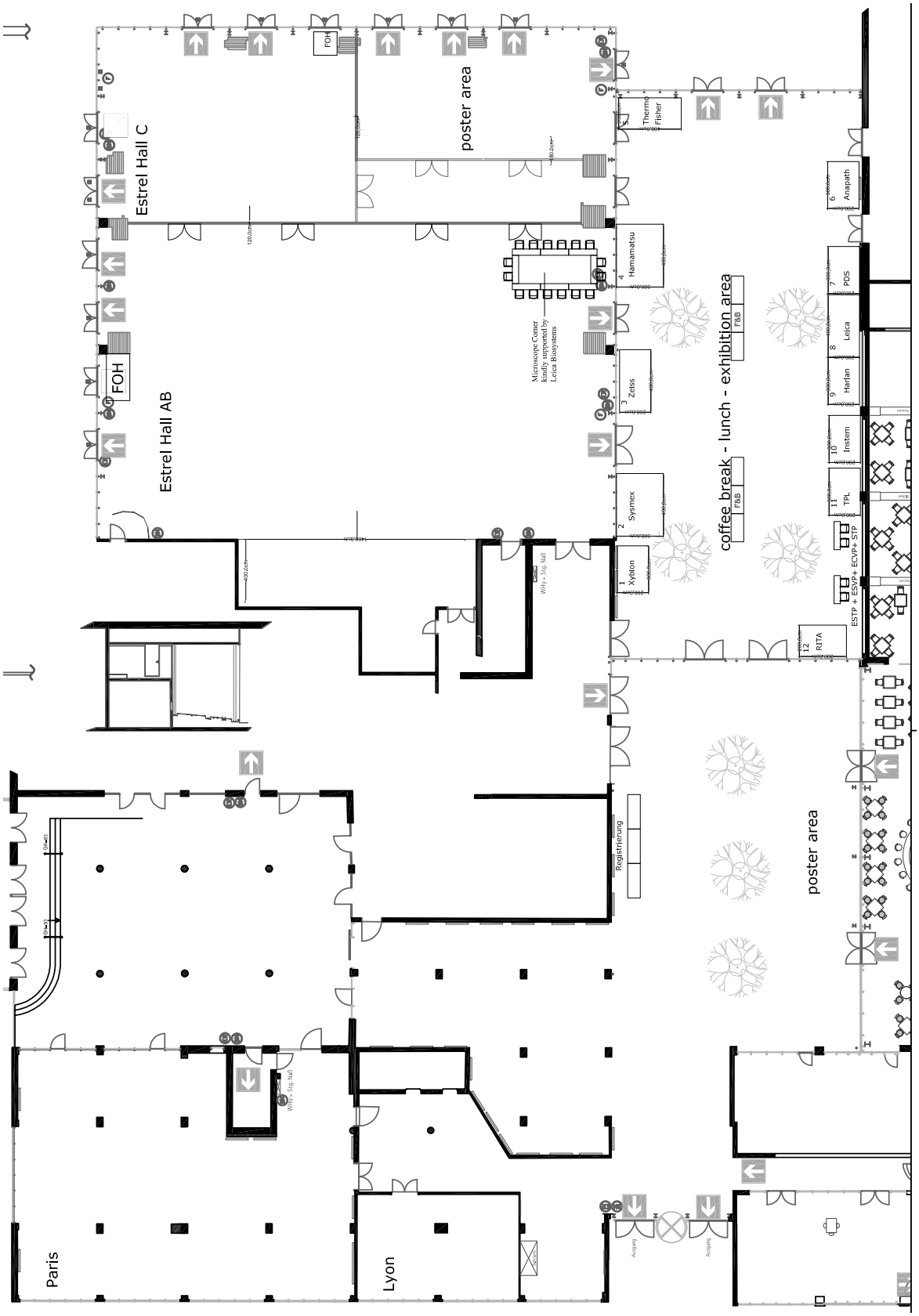
Room Plan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Paris
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Cutting Edge Pathology 2014
27th – 30th August

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Birgit Kittel, ESTP Journal Councillor, Novartis Pharma, Switzerland

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GlaxoSmithKline, UK

Zuhal Dincer, Coopted member for the Newsletter,
Novartis Pharma AG, Switzerland

Erio Barale-Thomas ESTP SFPT Representative, Johnson & Johnson, Belgium

György Selényi, Representative for the Hungarian Society of Toxicologic Pathology
Gedeon Richter Ltd., Hungary

Congress Information

1. Venue and General Information

Venue

The Congress will be held in the **„Estrel Hotel & Convention Center“** in Berlin Neukölln. All scientific sessions will take place in the main building of the hotel.

Public Transport

“S-Bahn” station: “Sonnenallee” Line: S42

Address:
Estrel Berlin
Sonnenallee 225
12057 Berlin
Germany

Official Language

English will be the congress official language.

Registration and Congress Secretariat

Registration will be offered during the Welcome Reception in the „Foyer Estrel Saal“ in the main building of the Estrel Hotel on the 27th August.

The registration desk will be located in the foyer of the Estrel Saal (main session room) between 27th – 30th August.

Opening Hours of the Congress Secretariat:

Wednesday, 27th August	07.30 a.m. – 09.00 p.m.
Thursday, 28th August	07.30 a.m. – 04.00 p.m.
Friday, 29th August	07.30 a.m. – 04.00 p.m.
Saturday, 30th August	08.30 a.m. – 11.00 a.m.

Phone: +49 176 238 65 665

Name Badges

Your name badge is your admission to the scientific sessions and to the coffee breaks and lunches. It should be worn at all times at the Congress venue.

Your name badge authorises you to use public transportation in central Berlin. Please take it with you when using the train, subway, tramp, or bus and present it upon request. However, please do not wear it visible outside of the Congress center.

Photography, Videotaping, Recording Policies

Photography of poster presentations is prohibited without the specific consent of the presenter(s)/author(s). Photography of exhibitor booths and/or equipment is prohibited without the specific consent of the exhibitor. Photography, videotaping, or recording of the Scientific Sessions is not permitted.

Internet Access

A laptop with internet access is at your disposal during business hours.

Free wireless internet access is available in your hotel room in the Estrel hotel. To connect your computer to the internet in the meeting area you'll need to purchase a code at the hotel reception.

Local Currency

German currency: **Euro**

You can exchange your foreign currency and traveller's cheques at German banks, exchange bureaus (called "Wechselstube" or "Geldwechsel" in German), airports, railway stations and major hotels.

German Banks are usually open Monday through Friday, 8:30 a.m. – 4:00 p.m. One of the fastest ways to withdraw cash is by using an ATM, called "Geldautomat" in German. They are ubiquitous in German cities and most can be accessed 24/7.

Before you leave home, make sure you know your PIN number. Visa, MasterCard, and American Express are usually accepted in Germany – but not everywhere.

Electricity

Electricity in Germany is 230 AC Volts, alternating at 50 cycles per second. If you travel to Germany with a device that does not accept 230 Volts at 50 Hertz, you will need a voltage converter. Sockets in Germany generally accept 1 type of plug: Two round pins. If your appliances plug has a different shape, you may need an adapter which is available at the convenience store in the basement of the Hotel.

Pharmacy

There are several pharmacies around the Estrel Hotel. They are called 'Apotheke' in German.

Emergency Calls

You should call 112 in case of emergency for ambulance, the police, or the fire brigade. It is a special emergency number you can call from any stationary or mobile phone.

International Calls

Dial 00 + country code + area code + phone number.
Germany country code is 49.

Messages

There will be a message board available close to the Congress registration desk.

Evaluation

Please help us to improve future congresses and fill out the evaluation form that you will find in your Congress documents. The form should be returned to the registration desk or mailed to us shortly after the Congress.

Anna-Lena Frisk:anna-lena.frisk@bayer.com

Gastronomy

Coffee and refreshments will be served during the breaks in the exhibition area of the foyer in front of the main session room „Estrel Saal". Times according to the programme.

You'll need your name badge as a ticket for coffee and refreshments.

Lunch will be provided during the lunch breaks on:
Thursday, 28th August
Friday, 29th August

For participants of the workshop, lunch will be provided on:
Wednesday, 27th August

2. Speaker Information

Digital files of your presentation should be handed to the technician in the lecture room where your talk is scheduled **at the latest during the break** before the start of your session. For the first morning session please hand in your presentation the day before. Please use a USB stick or CD-ROM. The use of your own computer is not recommended.

3. Poster Information

The two poster areas will be located in section C of the „Estrel Saal“ [ESTP posters (TP)] and in the „Passage“ in front of the Foyer [ESVP / ECVP posters (P)].

Poster viewing times and assignment of poster to topics are scheduled in the programme.

ESVP/ECVP posters scheduled in Poster Block I include the following topics:

Oncology

Degenerative Lesions

Fundamental Research

Immunology

Varia

These posters should be mounted on Wednesday between 4.30–5.00 p.m. and removed on Thursday between 07.00–07.30 p.m.

ESVP/ECVP posters scheduled in Poster Block II include the following topics:

Emerging and Other Infectious Diseases

Congenital Diseases

Zoo and Wildlife

Reptiles, Amphibians and Fish

Toxicology

Varia

These posters should be mounted on Friday between 7.15–7.45 a.m. and removed after the Closing Ceremony.

The ESTP posters (TP) will be on display during the entire Congress.

The ESTP poster sessions are scheduled on Thursday between 10.30–11.00 a.m., 1.15–2.00 p.m., and 4.00–4.30 p.m. The authors are kindly requested to attend their posters during these sessions for discussions and to answer questions.

The organisers take no responsibility for posters that will not be dismounted in time.

4. Industry Exhibition

The exhibition area will be located in the foyer of the „Estrel Saal“.

The industry exhibition provides information on the latest technologies and developments available in our field.

The exhibiting companies have a unique opportunity of effectively reaching their target customers. We value their support and believe that on-site discussions and experience between exhibitors and congress participants is invaluable for all. We cordially invite you to take full advantage of this wide spectrum of exhibitions.

An **exhibition quiz** is waiting for you: The documents needed for your participation will be handed out at the Congress counter. The winner will be announced and a prize awarded on Saturday during the coffee break.

5. Awards in Toxicologic Pathology

The BSTP Chirukandath Gopinath lecture on Thursday, 28th of August 02.15–03.15 p.m. will be held in lecture hall Paris.

The ESTP offers an award for an outstanding thesis in the field of Toxicologic Pathology.

French Society of Toxicologic Pathology (SFPT) Award for Best Poster

International Federation of Societies of Toxicologic Pathologists (IFSTP) Trainee Award

The award ceremony is scheduled on Friday, 29th August 03.00–03.15 p.m. after the session "Placenta of the Non-Human Primate" in lecture hall Paris.

5.1. Awards in Veterinary Pathology

The Journal of Comparative Pathology Plenary Award Lecture:

Prof. Dr. Wolfgang Baumgärtner, PhD (University of Veterinary Medicine Hannover):

Pathogenesis of Canine Spinal Cord Injury and Development of Cell-Based Treatment Options

Saturday, 30th Aug. 8.30–9.15 a.m., Lecture Hall A/B

ESVP-Awards for the **best oral presentations** and **best poster presentations** will be presented during the Closing Ceremony on Sat., 30th Aug., 1:00 p.m. All Congress participants will find voting vouchers in their Congress bags and are asked to place one vote per participant for one oral presentation and one poster, respectively. The vouchers should be deposited anonymously into the green box at the reception desk before Fri., 29th Aug. 6.00 p.m.

6. Additional Meetings

26.08.2014

10.00 a.m.–03.00 p.m.
Joint ESVP/ECVP and SOC Meeting
Planned for: Room Lyon

03.00–07.00 p.m.
ESVP Board Meeting
Planned for: Room 30310

04.00–06.00 p.m.
ESVP Council Meeting
Planned for: Room Lyon

27.08.2014

08.00 a.m.–05.00 p.m.
ECVP council meeting
Planned for: Room 30310

28.08.14

10.00 a.m.–12.00 p.m.
ESVP/ECVP SOC Meeting
Planned for: Room Lyon

01.00–02.00 p.m.
Satellite ESTP EC F2F Meeting
Planned for: Room 30310

29.08.2014

ESVP/ESTP Society Meeting
Time and Location tbd
See registration desk

10.00–11.00 a.m.
SFTP poster award Meeting
Planned for: Room Lyon

12.00 p.m.–01.00 p.m.
STP Presidents and Leaders Lunch
Planned for: Room 30310

7. Congress Dinner, Welcome Reception and Social Programme

The **dress code** of the Congress, including the Congress Dinner, is business casual.

Welcome Reception on 27th August:

In the evening of Wednesday, 27th August we would like to invite you to join us at the Welcome Reception in the Foyer of the „Estrel Saal".

Registration is open between 07.30 a.m. and 09.00 p.m. The Welcome Reception will start at 06.30 p.m. and is included in the Conference fee.

Conference Dinner on 29th August, 07.45 p.m.:

The Congress Dinner will take place in a typical German brewery in the center of Berlin. We would like to invite you to this informal evening to give you the chance to deepen the relationships with colleagues met during the Congress.

Address:
BHM Brauhaus Mitte GmbH
Karl-Liebknecht-Str. 13
10178 Berlin

In your Congress documents you will find a plan how to get there.
The Congress Dinner is included in the registration fees.

Social Programme:

Berlin Underworld:

Experience the history of Berlin from an unconventional perspective! Since 1997, the Berlin Underworlds Association has been offering regular tours into some of the most important underground structures in the city. Berlin was the center of the Third Reich and therefore one of the main targets for allied bombing during WW II. Among many other attractions, on these tours expert guides will lead you in exploring one of the few remaining bunkers, as it was left after the war.

Boat Tour:

From lush green spaces and historic buildings to glittering modern architecture, Berlin is a city that has it all. Today you can take in some of Berlin's best-loved sights and glimpse its lesser-known gems from a comfortable cruise ship with live narration. During the 3.5 hours journey you will discover more than 40 of Berlin's bridges.
Aboard the ship, a live guide will point out the city's most notable sights, weaving factual information with anecdotes that will bring the history of this storied metropolis to life. Among the trip's highlights are the Oberbaum Bridge, the Oberschleuse, the Technical Museum, Charlottenburg Castle, the Spreebogen, Bellevue Castle, The House of World Cultures, the Government Quarter, the Reichstag, Charité, Museum Island, the Berlin Cathedral, the Nikolaiviertel, The construction ground for the State Castle, and Mühlendammschleuse.

Berlin Museum of Medical History:

The Berlin Museum of Medical History is an institution of the Charité – University Medicine Berlin and is located in the former museum building of Robert Virchow´s Pathology Institute on the traditional grounds of the Charité.
The museum's permanent exhibition "On the Trace of Life" provides a path through medical history over the past 300 years. The presentation follows the ever-changing historical view of and into the body, finally arriving at the "recipient" of medicine, the patient, and the possibilities inherent in today's medicine. The heart of the museum is still the specimen hall, the core of which goes back to the collecting activities of Rudolf Virchow. Today, there are around 750 pathologic-anatomical wet and dry preparations on display in this area.
The ruin of the former Rudolf Virchow Lecture Hall, with its historic charm, presents a unique event location that has been designed for an unforgettable experience for guests from all over the world. The lecture hall of the pathological museum was destroyed towards the end of WW II by bombings. After the war, the building was barely refitted and almost forgotten.

Guided Tour to the Vet School:

Visit the Freie Universität Berlin Veterinary School: Excursion, 1h drive, Saturday 2.30–6.00 p.m.
You must register for the tour before Thu 28 th August, 6 p.m. at the Congress desk. Limited number of participants: 50.
The cost is 5 Euro per person.

8. Tips for your Berlin Tour

Gifts, Fashion & Design:

KaDeWe – Kaufhaus des Westens *is the largest department store in continental Europe*
Tauentzienstraße 21–24, 10789 Berlin

The Corner
Like Colette in Paris, where all the stars go when they are in Berlin
Französische Straße 40, 10117 Berlin

Galeries Lafayette
Greatest French and international brands and many French culinary specialties
Friedrichstraße 76–78, 10117 Berlin

Young Designer House
BIKINI BERLIN
Budapester Str. 38–50, 10787 Berlin

Bars, Food & Drinks:

Bar Reval
Eat tapas, Basque and Catalan in a modern canteen-like setting overlooking Görlitzer Park
Lübbener Straße 1, 10997 Berlin

Monsieur Vuong
Vietnamese restaurant with chic interiors in central Mitte
guidebook darling
Alte Schönhauser Straße 46, 10119 Berlin

... and don't forget to try a Berliner Currywurst!
Konnopke's Imbiß
Schönhauser Allee 44 A (under the subway-bridge), 10435 Berlin

Party & Clubbing:

Soda Club
Right in the middle of the Kulturbrauerei you will find an intimate club atmosphere
Schönhauser Allee 36, 10435 Berlin

Bath Boat / Badeschiff and MS Hoppetosse
Swimming, dining and dancing on the Spree
Eichenstraße 4, 12435 Berlin

Berghain
A 50 year old powerhouse were you're not just going out but clubbing professionally
Am Wriezener Bahnhof 20, 10243 Berlin

CUTTING EDGE PATHOLOGY

2nd Joint European Congress of the ESVP, ESTP and ECVP

27th – 30th August 2014, Berlin, Germany

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Keynote Lectures

KN1: LESSONS IN ENDOCRINE PATHOLOGY FROM HISTORY, ART, AND THE MICROSCOPE

Thomas J. Rosol

College of Veterinary Medicine, Ohio State University, Columbus, Ohio, USA

Endocrine glands were initially discovered as ductless glands that released an internal secretion or hormone. The word, hormone, is derived from a Greek term meaning to excite or arouse. The endocrine organs produce soluble hormones that include steroids, peptides and proteins, and amino acid derivatives. Hormones typically function in an endocrine manner, namely by distribution in the blood to end organs with specific receptors. Some hormones are transported in blood in free and protein bound forms, such as cortisol, vitamin D metabolites, and thyroid hormones. Although it is the free from of hormones that have biologic activity, protein binding increases the half-life of hormones in the blood and serves as a storage pool of hormone. Some hormones can be stored intracellularly and released when needed. A good example is the emergency hormone, calcitonin. Other hormones are produced on demand, such as steroid hormones, which are not stored but immediately diffuse from the cell of origin after synthesis. Hormones can also function in a paracrine (cell-to-cell), autocrine, and intracrine manner. Intracrine function is characterised by lack of secretion of a hormone and translocation of the hormone directly to the nucleus to regulate gene expression.

Endocrine organs in animals have diseases that can be genetic, developmental, inflammatory, degenerative, or proliferative in nature. There are often species and strain-specific predispositions to disease. In most cases there are spontaneous animal diseases in one or more species that can be used to model human endocrine disorders. However, there are notable exceptions, such as Graves' Disease of the thyroid gland. Graves' Disease is a condition of hyperthyroidism in humans that is caused by production of an immunoglobulin, which cross-reacts and activates the thyroid stimulating hormone receptor on thyroid follicular cells. This pathogenic mechanism has not yet been reported in animals, although experimental models of the disease have been developed.

Some endocrine diseases have been immortalised in art long before the diseases were understood. Endocrine disease in art dates from prehistory to the present time. Astute examination of works of art in museums or texts will reveal that iodine-deficient goiter in people and animals, gigantism, acromegaly, and dwarfism have been frequently captured in paintings, sculptures, and reliefs. In fact some cultures considered hyperplastic goiter a form of beauty. Many areas of the world are deficient in iodide, which resulted in a high incidence of hyperplastic goiter. Even today goiter is common in some countries and it is estimated that 200 million people have hyperplastic thyroid glands due to inadequate iodide intake or consumption of dietary compounds that inhibit thyroid synthesis.

Throughout history, endocrine disorders have been recognised in humans and animals for many years, but the mechanisms of disease were initially elucidated in the late 1800's and early 1900's. A landmark year was 1855. In that year, Thomas Addison, MD, first described the Disease of the Adrenal Capsules (Addison's Disease) (*Lancet*).

Initially there were four stages of endocrine discovery:

- Identification of a gland that has internal secretion(s)
- Detection of the internal secretion(s)
- Preparation of glandular extracts
- Isolation of pure hormone, identification of its structure, and its synthesis

These stages may have been accomplished years apart and required great dedication and labor by the scientists. For example, in 1961 Copp discovered the biological activity of calcitonin in dogs by perfusing the thyroid glands with a solution containing a high calcium concentration. In 1967 he demonstrated a similar biologic activity in the ultimobranchial glands

of chickens and fish. In 1968 Copp organised the collection 100 kg of ultimobranchial glands from 500,000 salmon in a fish packing plant in Vancouver. Salmon calcitonin was then biochemically purified, the amino acid sequence determined, and synthesised by Sandoz Pharmaceutical Co. (Basel) in the same year.

The pathogenic mechanisms that are responsible for abnormal endocrine function include:

- Primary and secondary hyperfunction of an endocrine gland
- Primary and secondary hypofunction of an endocrine gland
- Endocrine hyperactivity secondary to diseases of other organs
- Hypersecretion of hormone-like substances by nonendocrine tumours
- Failure of fetal endocrine function
- Endocrine dysfunction due to failure of target cell response
- Endocrine dysfunction resulting from abnormal degradation of hormone
- Iatrogenic syndromes of hormone-excess
- Exposure to or ingestion of substances or hormonal compounds that disrupt normal endocrine glands or axes

Endocrinology remains an active field of discovery to this day. For example, the clinical syndrome of humoral hypercalcaemia of malignancy (HHM) was reported in 1941 by Albright, but the causative hormone, parathyroid hormone-related protein (PTHrP), was not isolated and sequenced until 1987. Discovery was delayed because it was assumed that the cancers associated with HHM were secreting parathyroid hormone (PTH), since the biological activity mimicked the function of PTH. Since 1987, many endocrine, paracrine, autocrine and intracrine functions for PTHrP have been discovered for this hormone that was initially thought to be a cancer-related factor. Bone is now recognised as an endocrine organ since osteocytes produce and secrete FGF23, the hormone that regulates renal phosphate excretion. More recently, betatrophin, a new hormone produced by the liver that regulates beta cell number in the islets of Langerhans was reported in 2013 (Yi P et al, Cell). There are many hormones, hormone receptors, functions, and interactions yet to be discovered.

Comparative endocrinology and evolution of the endocrine system are areas of active investigation. Some endocrine organs, such as the pituitary and thyroid glands, are ancient and are found in the most primitive vertebrates in the oceans. Other glands have evolved more recently, such as the parathyroid glands, which are only present in amphibians and higher animals. The thyroid hormones and many of the steroid hormones are identical in humans, domestic animals, and primitive fish. The peptide hormones and their receptors have evolved over the years. Genome duplication events in fishes have resulted in enhanced hormone and receptor genetic variation in land vertebrates. Hormones that were thought to function only in fish have also been identified in higher vertebrates and humans.

It is recognised that hormonal feedback loops form complex integrated systems and counter-regulatory circuits that involve multiple organs and enable the body to maintain homeostasis even under perturbed conditions. In addition, the endocrine system communicates in both directions with the nervous and immune systems. Even though system regulation is multifaceted, histopathology of the endocrine organs can lead to conclusions about in vivo function. For example, the cytoplasmic area and nuclear:cytoplasmic ratio of parathyroid chief cells is proportional to hormone synthesis and secretion. Hypertrophy and hyperplasia of thyroid follicular cells is usually an indirect indicator of serum TSH concentrations. Flattened thyroid follicular cells is seen in animals that are euthyroid. Hypertrophy of pituitary basophils is an indicator of a lack of sex steroids. C-cell hyperplasia is seen in chronic hypercalcaemia. Functional endocrine tumours can often be identified by atrophy of the normal cells in the gland. Finally, immunohistochemistry is invaluable for identification of endocrine cells, especially in complex organs such as the pituitary gland. However, immunohistochemistry can often be misleading when trying to evaluate the functionality of endocrine cells. Cells that store abundant hormone will stain the most intensely, yet intense staining may not correlate with hormone synthesis and secretion. This is true for C cells. Normal C cells stain intensely for calcitonin because they have many intracellular secretory granules. C-cell tumours have less intense cytoplasmic staining for calcitonin because they secrete more hormone and store less. Cells may produce a high level of hormone, and have minimal immunohistochemical staining for the hormone because they rapidly secrete the hormone. This is the case with cancers that cause HHM. The cancer cells have abundant mRNA and a high level of PTHrP synthesis, but the cells stain lightly for PTHrP because it is rapidly secreted. In these cases, in situ hybridisation for hormone mRNA is a better indication of hormone synthesis.

The endocrine system and its diseases have a fascinating history, art repertoire, and pathology. Both the art and science of pathology enable the pathologist to have mechanistic insight into endocrine disease using the time honored tools of histopathology. Yet there remains a strong need for endocrine scientists to continue to discover new hormones and new interactions and regulatory mechanisms for the known hormones. Comparative endocrinology will continue to provide insight into the function and evolution of the endocrine system and its interactions with other body systems.

Suggested Reading:

Bentley PJ. (1998) Comparative Vertebrate Endocrinology. Cambridge University Press.

Capen CC and Martin SL. (1989) Mechanisms that lead to disease of the endocrine system in animals. Toxicol Pathol 17(2):234-249.

Medvie VC. (1982) A History of Endocrinology. MTP Press, Kluwer Boston Inc., Higham, Massachusetts.

Norris DO and Carr JA. (2013) Vertebrate Endocrinology, 5th Ed. Academic Press.

Rosol TJ, DeLellis RA, Harvey PW and Sutcliffe, C. (2013) Endocrine System. In: WM Hasckek, CG Rousseaux and MA Wallig (eds), Haschek and Rousseaux's Handbook of Toxicologic Pathology. Elsevier Inc., Academic Press, pp. 2391-2492.

Note: The Journal of Endocrinological Investigation (journal of the Italian Society of Endocrinology (SIE), Springer.com) often has a short feature on Endocrinology and Art that includes a classic painting or sculpture and a description with references.

Keynote Lectures

KN2: ANIMAL DISEASE CONTROL – DO WE STILL NEED PATHOLOGISTS?

Jens P. Teifke

Friedrich-Loeffler-Institute, Federal Research Institute for Animal Health, Germany

Pathology is one of the oldest disciplines in medicine and studies the mechanisms of disease based on morphological lesions and functional changes. Its aim in veterinary medicine is to advance our understanding of the pathogenetic causes and to help develop methods to prevent disease in animals and humans. Since antiquity and due to its fundamental importance, pathology has always been in the focus of the theoretical debate on health and illness. From this central position human, but also veterinary pathology act as bridging disciplines that link "cutting edge" biological research with clinical medicine and thus, eventually also with the diagnosis of diseases. In this context, veterinary pathologists play a key role in improving and protecting health and welfare not only of animals but also of humans. This underlines the crucial impact of veterinary pathologists for the human-animal-bond. With their abundant and vital expertise veterinary pathologists contribute to a wide range of topics in the field of public health: Monitoring and control of animal diseases, safeguard of animal welfare, diagnosis of new and emerging pathogens, assurance and improvement of food safety, establishment and development of animal models for human diseases, new drug development for animals and humans, epidemiological surveys and studies in domestic and wildlife animals, and this not only restricted to mammalians and vertebrates. Despite considerable advances that have been achieved in human and veterinary medical microbiology over the last decades, including sophisticated and extremely powerful, specific and sensitive molecular techniques such as proteomics and metagenomics, zoonoses, i.e. infectious diseases that are transmissible between animals and humans, are increasingly recognised as major sources of illness and death worldwide. The importance of animals as potential vectors for infectious diseases that affect human populations is growing. Globally, WHO estimates that 26% of human deaths are caused by infectious agents; 2/3 of human infections originate directly or indirectly from animals, and approximately 75% of recently emerging human infectious diseases are of animal origin.

Although various diagnostic manuals, including the golden standard of the O.I.E., prescribe gross pathology and histopathological techniques as mandatory for only very few reportable or notifiable infectious diseases, veterinary pathologists usually are, or should at least be among the first to encounter infectious disease outbreaks in animals. Necropsy including the open-view-approach of pathologists remains the state-of-the-art technology that opens the diagnostic window, especially in cases where collaborative work with various specialists including practitioners and clinicians, official veterinarians, epidemiologists, physicians, and microbiologists is needed. This lecture will demonstrate how veterinary pathologists in the past have played a critical role in promoting the knowledge of infectious diseases of public health impact. Prion diseases ('Mad Cow Disease'), infection with H5N1 influenza virus ('bird flu') or pandemic H1N1 influenza ('swine flu') are the most prominent examples for zoonoses that hit the news. Rabies in bats, African Swine Fever in wild boar, the emergence of new bunyaviruses in wild and domestic animals, differential diagnoses of vesicular diseases altogether with the implementation of new technologies such as microdissection, MALDI-imaging and automated quantitative image analysis applications will be targeted in the presentation and demonstrate that also in the future broad-based trained veterinary pathologists will be in an excellent position to be involved in the discovery of emerging and new infectious diseases, and remain a vital element in the multidisciplinary approach for animal disease control.

Keynote Lectures

KN3: UNDERSTANDING NANOTECHNOLOGY: AN EMERGING CHALLENGE FOR THE TOXICOLOGIC PATHOLOGIST

Ann F. Hubbs, Linda M. Sargent, Krishnan Sriram, Dale W. Porter, Liying Rojanasakul, Kara L. Fluharty, Vincent Castranova, and Robert R. Mercer

Centers for Disease Control and Prevention, Morgantown, USA
Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, 1095 Willowdale Rd, Morgantown, WV 26505

Nanotechnology is the technology which enables engineering in the nanoscale, a size range of less than ~100 nm. Nanotechnology products (NPs) have enormous and increasing economic impact. Importantly for toxicologic pathologists, NPs frequently have dimensions similar to subcellular structures. Since NPs are defined only by size, the number of potential products is virtually infinite (Hubbs *et al.*, 2013). The variety of NPs means that the potential effects are necessarily diverse. However, some features of the nanoscale can influence toxicity relative to micron-sized products with the same chemical composition.

Fundamentals of particle toxicology

In general, for a given material, particle toxicity increases as particle size decreases. The exception is the toxicity of liquid and highly soluble particles which largely depends upon mass because the chemical components rapidly dissolve. For particles of moderate solubility, nanoscaling generally enhances the dissolution rate. If the chemical components of those particles are toxic, this increases exposure rate and, thus, toxicity. For poorly soluble particles, nanoscaling increases surface area per unit mass that interacts with biological structures, generally increasing toxicity (Hubbs *et al.*, 2013). Particle biopersistence extends the duration of particle exposure, and, thus, enhances toxicity. Some inhalable fibers, such as asbestos, have well-established toxicity attributable to biopersistence combined with a high ratio of length to width, a feature known as high aspect ratio. Some new products of nanotechnology, such as the carbon nanotubes, combine biopersistence with a high aspect ratio and surface reactivity, suggesting that these nanoparticles may share toxicity features with asbestos (Donaldson and Poland, 2012). Finally, some particles contribute to altered immunologic and allergic responses by functioning as adjuvants or by enhancing the environmental dispersal of antigens (Beezhold *et al.*, 2003; Granum *et al.*, 2000). Recent studies suggest that it may be possible to intentionally manipulate the immune system using engineered particles to meet specific diagnostic and therapeutic needs (Moon *et al.*, 2012). A thorough understanding of nanoparticle toxicology is a necessary foundation for designing therapeutic NP that are optimised for desired biological effects and avoid unintended toxicities.

Intracellular and extracellular transport in the nanoscale

Many intracellular and extracellular translocation pathways have specific size limitations. For example, endocytosis can selectively translocate macromolecules and NPs into cells but may exclude material exceeding 100 nm in diameter. Extracellular circulation is similarly size limited. For example, entry into the lymphatic circulation is generally restricted to particles transported within phagocytes or to some extracellular NPs (Hubbs *et al.*, 2013). Importantly, the functional size of NPs *in vivo* is highly influenced by two factors 1) the agglomeration state, and 2) binding of biological material to the NP surface, creating a biomolecular corona. Two recent reviews summarise the current understanding of pathways which translocate NPs (Coppola and Caracciolo, 2014; Kettiger *et al.*, 2013). Specific targeting of these intracellular and extracellular pathways will be a key factor in the development of nanomedicine.

Lessons from the first generation products of nanotechnology

Recent studies reveal some concerning observations which are consistent with the principles of particle toxicology and enhanced intracellular and extracellular transport in the nanoscale. Inhaled multi-walled carbon nanotubes caused time-dependent accumulation in multiple tissues, pleural and nuclear penetration, as well as strong tumour-promotion

(Mercer *et al.*, 2013; Sargent *et al.*, 2014). Both multi-walled and single-walled carbon nanotubes also cause disruption of the mitotic spindle and aneuploidy *in vitro* at occupationally relevant doses. The mitotic spindle changes involved interaction between carbon nanotubes and the mitotic microtubules, chromatin and centrosomes (Sargent *et al.*, 2014). This suggests that some NPs interact with diverse subcellular structures, including those which control the fidelity of genetic information. Human exposures to these first generation NPs were frequently occupational. In order to protect workers, there are now strategies for the safe development of nanotechnology (Schulte *et al.*, 2014). A great deal can be learned from these criteria as strategies emerge for the safe development of nanopharmaceuticals which incorporate precise targeting of desired therapeutic effects while avoiding off-target toxicities.

Beezhold, D., Horton, K., Hickey, V., Daddona, J. and Kostyal, D. (2003). Glove powder's carrying capacity for latex protein: analysis using the ASTM ELISA test. *J Long Term Eff Med Implants,* **13,** 21-30.

Coppola, S. and Caracciolo, G. (2014). New views and insights into intracellular trafficking of drug-delivery systems by fluorescence fluctuation spectroscopy. *Ther Deliv,* **5,** 173-188.

Donaldson, K. and Poland, C. A. (2012). Inhaled nanoparticles and lung cancer – what we can learn from conventional particle toxicology. *Swiss Med Wkly,* **142,** w13547.

Granum, B., Gaarder, P. I., Eikeset, A., Stensby, B. A. and Lovik, M. (2000). The adjuvant effect of particles-importance of genetic background and pre-sensitisation. *Int Arch Allergy Immunol,* **122,** 167-173.

Hubbs, A., Porter, D., Mercer, R., Castranova, V., Sargent, L. and Sriram, K. (2013). Nanoparticulates. In: *Haschek and Rousseaux's Handbook of Toxicologic Pathology, Third Edition*, Wanda M. Haschek, Matthew A. Wallig, Brad Bolon and Ricardo Ochoa, Ed, Vol. II, Elsevier, pp. 1373-1419.

Kettiger, H., Schipanski, A., Wick, P. and Huwyler, J. (2013). Engineered nanomaterial uptake and tissue distribution: from cell to organism. *Int J Nanomedicine,* **8,** 3255-3269.

Mercer, R. R., Scabilloni, J. F., Hubbs, A. F., Battelli, L. A., McKinney, W., Friend, S., Wolfarth, M. G., Andrew, M., Castranova, V. and Porter, D. W. (2013). Distribution and fibrotic response following inhalation exposure to multi-walled carbon nanotubes. *Part Fibre Toxicol,* **10,** 33.

Moon, J. J., Huang, B. and Irvine, D. J. (2012). Engineering Nano- and Microparticles to Tune Immunity. *Advanced Materials,* **24,** 3724-3746.

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Schulte, P. A., Geraci, C. L., Murashov, V., Kuempel, E. D., Zumwalde, R. D., Castranova, V., Hoover, M. D., Hodson, L. and Martinez, K. F. (2014). Occupational safety and health criteria for responsible development of nanotechnology. *J Nanopart Res,* **16,** 2153.

Keynote Lectures

KN4: PATHOGENESIS OF CANINE SPINAL CORD INJURY AND DEVELOPMENT OF CELL-BASED TREATMENT OPTIONS

Journal of Comparative Pathology Plenary Award Lecture

Wolfgang Baumgärtner

Department of Pathology, University of Veterinary Medicine, Hannover, Germany

Spinal cord injury (SCI) is a devastating condition that may lead to severe disability and compromised quality of life. In humans, the incidence of SCI in Europe and North America is reported to be 15 to 39 cases per million. The precise incidence of SCI in dogs is uncertain, but spontaneous SCI represents one of the most common neurologic diseases. Intervertebral disk herniation (IVDH) is a frequent cause of SCI in dogs and occurs as a mixed compressive-contusive injury which shares similarities to its human counterpart.

The pathogenesis of SCI can be divided into 2 major processes: the initial mechanical injury (primary wave) and self-sustaining following processes referred to as secondary injury (secondary wave). The role of inflammation during secondary injury in SCI has been discussed controversially. Both detrimental as well as beneficial contributions of T cells, macrophages and cytokines have been described in experimental rodent SCI models. Especially a dominance of pro-inflammatory cytokines has been reported in experimental rodent SCI. However pathogenesis, cellular and cytokine responses may differ between rodents, dogs and humans. Indeed, several lines of evidence indicate that naturally occurring SCI in humans is dominated by a microglial response, whereas there is generally a marked lymphocytic response in experimental rodent SCI. Similarly, the astroglial responses in rodents differ from those in human SCI, with a delayed or even absent astrocyte reaction in humans. This indicates that translational models that bridge the gap between rodents and humans are needed. Spinal cord tissue of affected dogs is often available shortly after the initial insult; this contrasts with the generally long post-injury intervals in human SCI studies. The spontaneous and unpredictable occurrence of canine SCI might be regarded as a disadvantage. However, like the heterogeneous human patient population, the variability of canine SCI might also be viewed as more relevant to the human disease. SCI in dogs shares many similarities with its human counterpart including mechanics of lesion initiation, location, development, and similarities in tissue size and neuro-anatomy. Summarised, dogs may serve as a bridging model for future transplantation studies as a highly needed translational model. Thereby, results may be beneficial for the patient "dog" by providing new and innovative treatment options and for humans by relying on clinical data obtained from a highly suitable translational model.

In order to further substantiate the role of canine SCI as a translational model it is necessary to characterise spinal cord lesion development in detail by using histology, immunohistochemistry and profound molecular analysis. This information is also essential to develop new treatment strategies. Furthermore, the use of cell transplantation as one treatment option requires detailed knowledge about glial cell populations in the translational model. Moreover, suitable in vitro systems are needed to investigate selected aspects of spinal cord injury pathogenesis or to evaluate treatment approaches in a pre-clinical set-up. Lastly, before going into clinical trials in dogs, some aspects need to be studied in rodent models allowing an in-depth evaluation of the benefits or possible complications of the envisioned approaches for both humans and dogs.

Acute spinal cord lesions of due to IVDH were characterised by a variable degree of necrosis, haemorrhage, scant inflammation and axonopathy. In contrast, macrophage activation, axonopathy, myelin loss and immune processes were prominent in subacute lesions. The inflammatory response in subacute canine SCI was largely restricted to resident immune cells as demonstrated by activation of major histocompatibility complex class II (MHC II) expressing microglia/macrophages. By quantitative polymerase chain reaction, an up-regulation of pro-inflammatory cytokine genes (i.e. of interleukin 6 [IL-6] and IL-8 with a trend towards an up-regulation of tumour necrosis factor) in acute canine SCI was noticed, whereas expression of neuroprotective cytokines (e.g. IL-10) remained unchanged or were noticed later (transforming growth factor-β).

Axonopathy, consisting of swollen axons and spheroids, respectively, represents a frequent finding in canine IVDH. Immunohistochemically, enhanced axonal expression of beta-amyloid precursor protein, non-phosphorylated neurofilament and growth-associated protein-43 was detectable in the epicenter of acute canine SCI. These changes showed a cranial and caudally accentuated spatial progression in the subacute disease phase. Real-time quantitative polymerase chain reaction of naturally traumatised tissue revealed a temporarily distinct dysregulation of the matrix metalloproteinases (MMP)-2 and MMP-9 with a dominating expression of the latter.

Organotypic spinal cord slice cultures represent a highly interesting in vitro model for addressing the roles of resident glial cells during the secondary injury phase. In contrast to dissociated tissue cultures, slice cultures have the advantage of representing preserved organotypic CNS architecture and they are alternatives to live animal experiments. Because of a lack of blood supply, morphologic and molecular immunologic reactions are restricted to those of the resident, rather than infiltrating immune cells in this system. In organotypic canine spinal cord slices, a similar activation of MHC II positive microglia as observed in in vivo traumatised spinal cord lesions was noticed. In addition, a prolonged up-regulation of inflammatory cytokines were observed. This substantiates that resident rather than infiltrating cells play major roles in the post-injury immune response.

Suitable cell candidates for transplantation include a variety of glial cells including oligodendrocyte precursor cells (OPCs) and Schwann cells. However, transplantation of a murine OPC line, termed BO-1, expressing the enhanced green fluorescent protein (eGFP) in ethidium bromide induced lesions in mice resulted in an uncontrolled neoplastic growth. This indicated that this cell line does not represent a suitable option for an exogenous cell transplantation therapy. Interestingly, certain canine glial cells, which are promising candidates for cell-based therapies, exhibit primate cell like properties, whereas rodent glia differ from their human counterpart in their cultural requirements and phenotypes. To further explore the potential of canine glial cells for transplantation, they have been investigated using microarray assays, in vitro studies and a preclinical rodent transplantation trial.

Schwann cells (SCs), olfactory ensheathing cells (OECs), and central nervous system Schwann cell-like glia (SG) represent a group of p75 neurotrophin receptor (NTR)-positive cells with pro-regenerative capacities. These cells are subjects in experimental transplantation-based therapies of spinal cord trauma and are especially suitable for similar treatment concepts in dogs. The transcriptomes of canine OECs, SCs, SG and fibroblasts (FBs) revealed a lack of transcriptional differences between OECs and SG, a high similarity of OECs/SG to SCs, and a marked difference of SCs and OECs/SG towards FBs. Furthermore, cell type-specific biomarkers were detected. Thereby aquaporin 1 (AQP1) and stimulator of chondrogenesis 1 (SCRG1) were predicted to be the most powerful biomarkers to distinguish SCs from OECs/SG. This was confirmed by immunofluorescence. A higher expression of SCRG1 was noticed in OECs and SG, and conversely a prominent up-regulation was found for AQP1 in SCs in vitro. Conclusively, OECs/SG seemed to represent a uniform cell type, which is highly related to SCs.

Similarly, olfactory mucosa (OM)-derived OECs are attractive candidates for an autologous cell transplantation-based therapy of nervous system injury. However, defining the regenerative capacity of OM-derived OECs is impeded by the fact that cell cultures used for transplantation may contain significant amounts of contaminating trigeminal nerve SCs that escape identification by sharing in vitro expression of OEC markers. Therefore various protocols to obtain purified cell populations have been developed. Interestingly, neurite-growth of neonatal rat dorsal root ganglion (DRG) neurons co-cultured with canine OM-OECs, olfactory bulb (OB)-OECs, and fibular nerve (FN)-SCs was significantly higher in the presence of OECs than of SCs. Similarly, a key controversy has been whether transplanted OECs can properly remyelinate axons, or whether the observed remyelination is caused by contaminating SCs in cell preparations by standardised protocols. To address this issue the efficacy for remyelination of purified canine OB-OECs, OM-OECs and SCs was compared after transplantation into a persistently demyelinated lesion in the rat dorsal funiculus. Comparative histological analysis indicated that purified canine OECs (mucosal and bulb) and SCs survived the transplantation procedure and contributed to subsequent remyelination. While all three cell types remyelinated spinal cord axons, OB-OEC and SC transplantation resulted in extensive peripheral-like myelin within the spinal cord, whereas OM-OECs displayed only modest remyelination. An in vitro migration assay indicated that OM-OECs also migrated less than OB-OECs and SCs.

The obtained in vivo and in vitro data about the pathogenesis of canine SCI and the detailed characterisation of glial cells potentially suitable for transplantation resulted in a clinical trial. Diseased dogs suffering from chronic SCI due to disk protrusion received after conventional surgery Schwann cells derived from the dorsal root of the spinal nerve for autologous transplantation after their in vitro cultivation. Conclusively, both in vivo and in vitro research has substantiated that the dog represents a promising translational animal species, which completes experimental rodent studies and might allow extrapolations to the human disease. Simultaneously, in such a scenario new treatment concepts will also be developed and approved for the benefit of the canine species.

Keynote Lecture

KN5: PREDICTING DISEASE OUTCOME AND THERAPY RESPONSE IN CANCER: MORPHOLOGY, IMMUNOHISTOCHEMICAL MARKERS OR MOLECULAR SIGNATURES – WHAT TO CHOOSE?

Sven Rottenberg

Vetsuisse Faculty, University of Bern, Switzerland

For the treatment of cancer patients, pathologists play a crucial role for the diagnosis and for predicting disease outcome (prognosis). Once a clinician is faced with a cancer patient whose prognosis is poor – usually due to the presence or risk of cancer metastases – the question of systemic therapy arises. Many human patients are then treated with a toxic cocktail of drugs. Most will experience the side effects but only in some patients tumours will shrink substantially or even be eliminated. Which patients will benefit from conventional chemotherapy and which patients will only develop side effects is usually unpredictable. To better predict therapy outcome is therefore a major goal of molecular pathology. It provides an opportunity for pathologists to apply their skills even further and help clinicians to identify the optimal treatment to improve patient survival and quality of life.

Although chemotherapy is less common to treat animal patients, the number of pet owners who want their veterinarians to apply systemic anti-cancer therapy steadily increases. Moreover, next to classical chemotherapy a plethora of more tailored anti-cancer drugs has become available, and several of these are now entering veterinary medicine. It is therefore inevitable that veterinary pathologists are involved in the question whether a specific anti-cancer drug will be helpful for an individual animal.

Techniques to predict therapy outcome are manifold: in addition to morphology and in situ analyses, genome- and proteome-wide profiles have been correlated with therapy outcome. But are they really helpful? Although prognostic signatures flourish, predictive signatures are rather elusive. Using breast cancer in humans and mammary tumours in animals as an example, I will discuss why it is difficult to derive meaningful predictive signatures. Approaches that should be able to yield predictive markers for optimising cancer treatment will be delineated.

Joint Interactive Slide Session
Toxicopathology-related and Diagnostic Pathology Cases

ESTP Case 1: Thierry Flandre

Novartis Pharma AG, Basel, Switzerland

CASE TITLE:	DEGENERATIVE AND INFLAMMATORY LESIONS ASSOCIATED WITH AN IMMUNOSUPPRESSIVE DRUG IN CYNOMOLGUS MONKEY
ABSTRACT:	During a *4-week* toxicity study in cynomolgus monkeys with an immunosuppressive monoclonal antibody, lymphoid depletion as an expected pharmacological effect was observed, eg decreased cellularity of lymphoid follicles with absence of germinal center development. In addition, there were non-expected findings as well, like diarrhoea, which was noted at the end of the study in few treated animals. At microscopy, degenerative and inflammatory changes were found in the heart, kidney and lung in all treated animals. Crypt hyperplasia with neutrophilic infiltration in the colon was also observed in diarrhaeic animals. With biologic compounds causes of toxicity are limited and mostly related to immune complex (i.e. due to Anti-Drug Antibody), exaggerated pharmacology (i.e. cytokine modulation) and/or off-target toxicity. In case of an immunosuppressive drug, opportunistic infections also need to be taken into consideration.
Label on histoslides	No slides provided, pictures only: ESTP Case 1
ANIMAL(S):	
Species, breed	Cynomolgus monkey, Mauritius
Sex	Male and Female
Age	2-4 years
Study type	4-week intravenous toxicity study
Treatment	Monoclonal antibody
Clinical findings	Diarrhoea
Organ(s)	Kidney, heart, lung and colon
Gross finding(s)	–
Staining	HE, IHC
WHAT'S YOUR DIAGNOSIS?	

Joint Interactive Slide Session
Toxicopathology-related and Diagnostic Pathology Cases

Diagnostic Case 1: Frauke Seehusen

Department of Pathology, University of Veterinary Medicine Hannover, Germany.

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CASE TITLE:	INFILTRATIVE MASS AFFECTING THE RIGHT HINDLIMB OF A CAT
ABSTRACT:	An infiltrative mass was detected in the right tarsal region of a cat.
Label on histoslides	E 6005/12 C (diagnostic case 1)
ANIMAL(S):	Cat
Species, breed	Cat, Domestic short hair
Sex	Male (castrated)
Age	5 years
Study type	–
Treatment	–
Clinical findings	–
Organ(s)	Subcutis, mass affecting the right tarsal region, FFPE
Gross finding(s)	Distal to the tarsal joint, a multi-lobulated, reddish-white mass measuring approximately 5 by 5 by 4 cm was found in the subcutis. The mass surrounded the metatarsal bones on the dorsal, medial and plantar aspects of the limb and infiltrated the extensor tendons. The joint was not affected.
Staining	HE

WHAT'S YOUR DIAGNOSIS

Joint Interactive Slide Session
Toxicopathology-related and Diagnostic Pathology Cases

ESTP Case 2: Birgit Kittel

Novartis Pharma, Basel, Switzerland

CASE TITLE:	UNUSUAL FINDING IN THE GLANDULAR STOMACH OF A YOUNG MALE WISTAR RAT
ABSTRACT:	In a two week pilot oral gavage study male Han-Wistar rats, approximately 12 weeks of age, were daily dosed with compound or vehicle only (Methyl cellulose, Polysorbate, in aqueous solution). Clinical observations, body weight and food consumption, haematology, clinical chemistry and urinalysis, gross pathology examinations and organ weight determinations were performed. Microscopic examinations were conducted on a standard list of organs and tissues. Administration of the compound was generally well tolerated; no significant in-life findings were neither observed nor significant off-target toxicicty. Upon microscopic examination, an unusual finding was noted in the standard section of the glandular stomach of a single rat. The change was characterised by well circumscribed in-growing epithelium that was present in the standard histologic section of the pyloric area; no macroscopic finding was noted. No other changes in the GI tract were observed in any of the rats. The case, its challenge of diagnosis and potential differential diagnoses will be discussed in the context of current nomenclature and available historical data in an interactive session.
Label on histoslides	No slides provided, pictures only: ESTP Case 2
ANIMAL(S):	Rat
Species, breed	IGS Wistar Hannover Rat; Crl: WI(Han)
Sex	Male
Age	Ca. 12 weeks
Study type	Pilot toxicity study
Treatment	2 week oral gavage
Clinical findings	–
Organ(s)	Glandular stomach
Gross finding(s)	–
Staining	HE
WHAT'S YOUR DIAGNOSIS?	

Joint Interactive Slide Session
Toxicopathology-related and Diagnostic Pathology Cases

Diagnostic Case 2: Anja Ostrowski, Angele Breithaupt

Freie Universität Berlin, Germany. e-mail: anja.ostrowski@fu-berlin.de

CASE TITLE:	MASS IN THE NASAL CAVITY OF A WEST HIGHLAND WHITE TERRIER DOG
ABSTRACT:	An intact male, 10 years old West Highland White Terrier dog developed recurrent seizures with ptyalism and was euthanised in a status epilepticus.
Label on histoslides	S55/2014 (diagnostic case 2)
ANIMAL(S):	Dog
Species, breed	Dog, West Highland White Terrier
Sex	Male (intact)
Age	10 years
Study type	–
Treatment	–
Clinical findings	–
Organ(s)	Mass in the nasal cavity, FFPE
Gross finding(s)	A beige, soft mass of approximately 2.1 x 1.9 x 1.6 cm in size was mainly located within the nasal cavity (bilateral, centro-dorsal), partially compressing and replacing the nasal turbinates.
Staining	HE
WHAT'S YOUR DIAGNOSIS	

Joint Interactive Slide Session
Toxicopathology-related and Diagnostic Pathology Cases

ESTP Case 3: Thomas Lemarchand

Covance, France

CASE TITLE:	PULMONARY LESIONS IN RABBITS
ABSTRACT:	A 16 week old New Zealand White female rabbit was found dead after dosing on day 27 of a 37-day long sub-chronic toxicological study. On macroscopic examination, few dark red areas were observed on all lung lobes.
Label on histoslides	8262273 34 13 1 & 36 1, ESTP Case 3
ANIMAL(S):	rabbit
Species, breed	New Zealand White
Sex	female
Age	16 week old
Study type	Subchronic toxicology
Treatment	oral
Clinical findings	–
Organ(s)	Lung
Gross finding(s)	Red areas
Staining	HE

WHAT'S YOUR DIAGNOSIS?

Joint Interactive Slide Session
Toxicopathology-related and Diagnostic Pathology Cases

Diagnostic Case 3: Paola Roccabianca

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CASE TITLE:	CORSO DOG WITH PROGRESSIVE LAMENESS
ABSTRACT:	The patient was evaluated for moderate lameness of the right hind limb. Radiographs of the coxofemoral and stifle joints were interpreted as normal. Lameness worsened and 6 months after initial presentation the dog was reevaluated. A painful swelling measuring 2 cm in diameter was present on the proximal tibial region. Based on radiographic findings osteomyelitis or osteosarcoma were suspected. At 18 months of age the dog was referred for a second opinion. The dog was now severely lame and a painful soft tissue swelling (10 cm by 5 cm) of the right proximal tibia was found. Radiographs revealed a lytic lesion associated with a pathological fracture of the tibial diaphysis and with a disorganised periosteal reaction. A free-hand fine-needle aspiration biopsy was performed with a 21-gauge needle. Fluid (2 mL) was collected and submitted for cytology.
Label on histoslides	PP1008/01 (diagnostic case 3)
ANIMAL(S):	Dog
Species, breed	Dog, Cane Corso
Sex	Female (intact)
Age	11 months
Study type	–
Treatment	The dog was discharged after the first examination with carprofen (2 mg/kg per os every12 hours for 10 days). No other therapies were given.
Clinical findings	–
Organ(s)	Cytological specimen, fine needle aspiration, tibial diaphysis
Gross finding(s)	Bone lysis after 6 month history of lameness.
Staining	May-Grünwald Giemsa staining
WHAT'S YOUR DIAGNOSIS	

Joint Interactive Slide Session
Toxicopathology-related and Diagnostic Pathology Cases

ESTP Case 4: Alessandro Piaia

Novartis Pharma, Basel, Switzerland

CASE TITLE:	Eosinophilic crystals in the rat and mouse lungs: comparing their pathogenesis, microscopic appearances and frequency.
ABSTRACT:	Eosinophilic crystals, which occur as a common background change in the rat and mouse, have been well characterised in the literature. Despite of their common occurrence, they are relatively harmful to the lung cells and might lead to complications such as inflammation in the lung and/or mimic treatment-related effects. In addition, particularly in the knock-out mice, the condition can limit the survival rate and complicate the development of animal models used in research. Similar crystals have also been observed in other mammals. In human they have been related to crystallised eosinophil protein, such as lysophospholipase (Charcot-Leyden crystals). Rat and mouse crystals do not share the same origin as human, and interestingly, considering the close evolutionary relationship of these two rodent species, they do not have either the same origin between them. This case will compare the differential pathogenesis, microscopic appearance and the frequency of eosinophilic crystals in the rat and mouse and discuss what similar condition was described for human in the literature. The cases will be also discussed in the context of current INHAND nomenclature and available historical data in an interactive session.
Label on histoslides	No slides provided, pictures only: ESTP Case 4
ANIMAL(S):	Rat, Mouse
Species, breed	various
Sex	
Age	various
Study type	Inhalation toxicity study and mouse knockout characterisation
Treatment	various
Clinical findings	–
Organ(s)	Lung
Gross finding(s)	–
Staining	HE

WHAT'S YOUR DIAGNOSIS?

Joint Interactive Slide Session
Toxicopathology-related and Diagnostic Pathology Cases

Diagnostic Case 4: Leslie Bosseler

Department of Pathology, Bacteriology and Avian Medicine – University of Ghent, Belgium.

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CASE TITLE:	ACUTE LIVER FAILURE IN AN EXPERIMENTAL BEAGLE
ABSTRACT:	The patient that was used as a control dog in an experimental study suddenly developed symptoms of acute liver failure with fever and died the following day. The dog had been on phenobarbital (16 mg/kg) for 6 weeks and lived together with other dogs. All the dogs had access to an inside and outside enclosure. On necropsy, severe icterus, multiple haemorrhages, an enlarged, friable liver, splenomegaly and enlarged abdominal lymph nodes were seen, as well as a gastric foreign object (rubber toy).
Label on histoslides	F6361-2 (diagnostic case 4)
ANIMAL(S):	Dog
Species, breed	Dog, Beagle
Sex	Male (castrated)
Age	6 years
Study type	–
Treatment	Phenobarbital (16 mg/kg) for experimental study. After onset of symptoms, Amoxycillin-clavulanic acid, blood transfusion, oxygen, pain medication
Clinical findings	Acute onset of: lethargy, anorexia, fever, icterus, tachycardia, anemia, epistaxis
Organ(s)	Liver, FFPE
Gross finding(s)	Large, friable, moderately pale liver with an increased lobular pattern, icterus, hepatic and mesenteric lymphadenopathy, multifocal haemorrhages, subcutaneous oedema, sero-haemorrhagic ascites, foreign objects in the stomach (pieces of a rubber toy).
Staining	HE
WHAT'S YOUR DIAGNOSIS	

Joint Interactive Slide Session
Toxicopathology-related and Diagnostic Pathology Cases

ESTP Case 5: Vanessa L. Schumacher

Roche Innovation Center Penzberg, Germany

CASE TITLE:	LIVER FINDINGS IN RAG2-/-γC-/- IMMUNODEFICIENT MICE
ABSTRACT:	Two 8-10 week-old Rag2-/-yc-/- mice on a Balb/c background in the 17th day of gestation were submitted for pathology analysis after being found dead in the cage 2 days after delivery to the animal facility. On gross examination, the liver and kidneys were diffusely pale tan in colour, and the gastrointestinal tract was nearly empty of contents. Both mice were pregnant. Histologic findings were present in the liver and kidney of both mice. In the liver, there was diffuse vacuolar change of hepatocytes characterised by one (macrovesicular) or multiple (microvesicular) discrete clear cytoplasmic vacuoles that occasionally displaced the nuclei to the edge of the cell. In the kidney, there was mild, multifocal, discrete vacuolar change in the cytoplasm of renal proximal tubules.
Label on histoslides	No slides provided, pictures only: ESTP Case 5
ANIMAL(S):	
Species, breed	2 Rag2-/-yc-/- mice on a Balb/c background
Sex	Female
Age	8-10 weeks old
Study type	–
Treatment	–
Clinical findings	Found dead 2 days after delivery to facility. The mice were in the 17th day of gestation.
Organ(s)	Liver, kidney
Gross finding(s)	The liver and kidneys were diffusely pale tan in color, and the gastrointestinal tract was nearly empty of contents. Both mice were pregnant.
Staining	HE

WHAT'S YOUR DIAGNOSIS?

Joint Interactive Slide Session
Toxicopathology-related and Diagnostic Pathology Cases

Diagnostic Case 5: Sara Malberg, Manfred Henrich, Christiane Herden

Institute of Veterinary Pathology, Justus-Liebig-University Giessen, Germany.

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CASE TITLE:	UNEXPECTED LESIONS IN DYSPNOEIC IMPORTED PUPPIES
ABSTRACT:	An unvaccinated bitch with a litter of 6 puppies had been imported from Croatia. 3 puppies exhibited severe dyspnoea and mild diarrhoea.
Label on histoslides	Diagnostic case 5 brain, diagnostic case 5 liver
ANIMAL(S):	Dog
Species, breed	Dog, Beagle-Mix
Sex	Male (intact)
Age	8 weeks
Study type	–
Treatment	Antibiotics (amoxicillin + clavulanic acid), NSAID (metamizole), secretolytic drugs (bromhexine, N-acetyl cysteine), inhalation with NaCl
Clinical findings	Clinical examination in all affected puppies revealed increased respiratory rate, laryngeal oedema with inspiratory stridor and severely abnormal respiratory sounds. In diagnostic X-ray imaging of one of the puppies a moderate laryngeal oedema and an increased interstitial lung density were visible. Worsening of dyspnoea with development of cyanosis despite of treatment led to euthanasia of all affected puppies; 2 of them were presented for necropsy.
Organ(s)	Brain, liver, FFPE
Gross finding(s)	Laryngeal and perilaryngeal oedema, moderate to severe; Lung with multiple dark red foci of consolidation, preliminary diagnosis of pneumonia; Liver with soft and friable parenchyma and acute congestion; Enteritis, catarrhal, mild, acute, diffuse with lymphadenitis simplex of mesenterial lymph nodes and mild nematode infestation.
Staining	HE

WHAT'S YOUR DIAGNOSIS

Joint Interactive Slide Session
Toxicopathology-related and Diagnostic Pathology Cases

ESTP Case 6: Silke Treumann

BASF SE, Germany

CASE TITLE:	UNUSUAL RENAL NEOPLASTIC LESIONS IN YOUNG WISTAR RATS
ABSTRACT:	Tumours, and hyperplasias, of amphophilic-vacuolar tubular phenotype in the kidneys were increasingly observed in studies at BASF SE in the last two years using Wistar-Han rats (Crl:WI[Han]). Tumours or hyperplasia were noted in individual animals of both sexes, including animals from control and treated groups. The studies were of different types ranging from 4 weeks up to 3 months.
Label on histoslides:	01R025-7-3A, ESTP Case 6
ANIMAL(S):	Rats
Species, breed	Wistar (Crl:WI[Han])
Sex	Both male and female
Age	10 weeks to 20 weeks
Study type	4 week feeding, mode of action, inhalation and reproduction studies
Treatment	Control and single cases in various treatment groups
Clinical findings	–
Organ(s)	kidney
Gross finding(s)	Cyst, mass, focus or none
Staining	HE
WHAT'S YOUR DIAGNOSIS?	

Joint Interactive Slide Session
Toxicopathology-related and Diagnostic Pathology Cases

Diagnostic Case 6: Andreas Beineke, Frauke Seehusen

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CASE TITLE:	UNUSUAL PROLIFERATION IN THE SKIN OF A YOUNG PUG DOG
ABSTRACT:	A small mass was found in the haired skin of a pinna in a 5 month-old pug dog.
Label on histoslides	E 2936/13 A1 (diagnostic case 6)
ANIMAL(S):	Dog
Species, breed	Dog, Pug dog
Sex	Male (intact)
Age	5 months
Study type	–
Treatment	–
Clinical findings	–
Organ(s)	Haired skin, ear, FFPE
Gross finding(s)	A small mass was found in the haired skin of a pinna.
Staining	HE
WHAT'S YOUR DIAGNOSIS	

Joint Interactive Slide Session
Toxicopathology-related and Diagnostic Pathology Cases

ESTP Case 7: Yu-Ling Chen

National Laboratory Animal Center, Taiwan

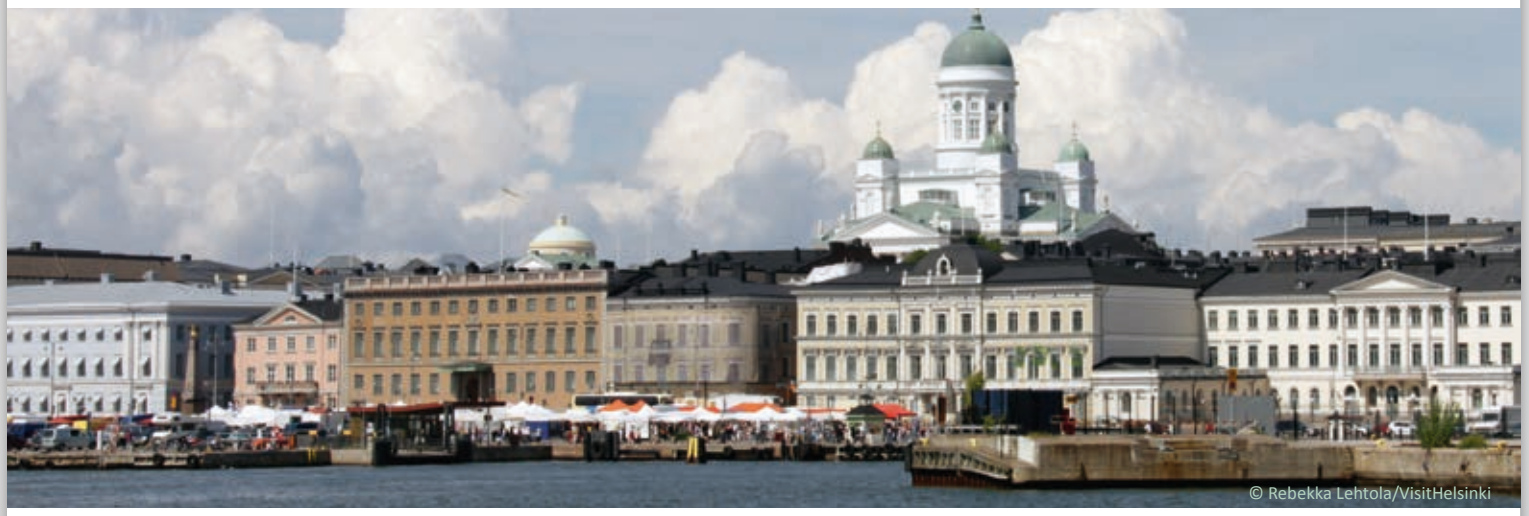
CASE TITLE:	PROLIFERATIVE LESION OF AN AGED RAT IN THE SPINAL CORD
ABSTRACT:	Here we report the gross, histologic, and immunohistochemical characteristics of a naturally occurring peripheral nerve neoplasm in a WKY rat. The inbred WKY rat is the normotensive control of the spontaneously hypertensive rat (SHR), which both are derived from the same ancestral Wistar rat. Paralysis of the hind limbs was found in a one-year-old female WKY rat from the breeding colony and sent for necropsy examination. A swollen and pale mass with spinal compression was found in the lower thoracic region. The light microscopic findings and results of additional investigations will be presented during presentation.
Label on histoslides	Yu-Ling Chen National Laboratory Animal Center Taiwan, ESTP Case 7
ANIMAL(S):	Rat
Species, breed	Wistar-Kyoto rat
Sex	Female
Age	1-year-old
Study type	NA
Treatment	–
Clinical findings	Hind limbs paralysis
Organ(s)	Spinal cord
Gross finding(s)	A swollen and pale mass with spinal compression was found in the lower thoracic region
Staining	HE and IHC

WHAT'S YOUR DIAGNOSIS?

THE EUROPEAN SOCIETY OF VETERINARY PATHOLOGISTS 33RD ANNUAL MEETING & THE 26TH ANNUAL MEETING OF EUROPEAN COLLEGE OF VETERINARY PATHOLOGISTS

will take place jointly with the meeting of Nordic Society of Veterinary Pathology on

the 2nd to 5th of September 2015, in Helsinki, Finland



VENUE

The meeting will be held in Helsinki at the Marina Congress Center in Katajanokka. Marina Congress Center is a congress venue with the latest meeting technology located at the waterfront in the center of the city.

PROGRAM

Wednesday Sept 2nd, Registration, Get together and University of Helsinki Reception
Thursday Sept 3rd, Full day conference, City of Helsinki Reception
Friday Sept 4th, Full day conference, Congress dinner
Saturday Sept 5th, ½ day ESVP-ECVP conference, ½ day NSVP annual meeting with mystery slide sessions

PRE-CONFERENCE MEETING ON THE 1ST & 2ND OF SEPTEMBER: a course for anatomic pathologists on the cytology of hematopoietic disorders and tumor masses. Lecturers: Prof. Mary Anna Thrall USA and Prof. Donald Meuten USA. The course is organized by Nordic Society of Pathology/Evira chapter. (Inquiries: Veera Karkamo veera.karkamo@evira.fi)

POST-CONFERENCE MEETING: one and half day C. L Davis symposium 5th and 6th of September.
(Inquiries: Liz McInnes efmcinnes@aol.com)

We are looking forward to seeing you in Helsinki and welcome you to join us for the ESVP-ECVP 2015 Meeting on behalf of the ESVP and ECVP Local Organizer Committee:

Prof Antti Sukura, University of Helsinki
Prof Marjukka Anttila, Finnish Food Safety Authority Evira
Clinical teacher Pernilla Syrjä, University of Helsinki

Conference agency Konffa Ltd, e-mail: vp@konffa.fi,
tel: +358 10 2926 500.

See more info at www.esvp.eu and/or www.esvpecvp.fi

Sightseeing trips and guidances in Helsinki, please visit:
http://www.visithelsinki.fi/en/whats-on/sightseeing-trips-and-guidances

ESVP ecvp European College of Veterinary Pathologists UNIVERSITY OF HELSINKI Evira

List of Contributions and Abstracts other than Key Note Lectures

Invited Talk

I1: PATHOSURVEILLANCE – PAST, PRESENT AND FUTURE

Sandra Scholes

Animal Health Veterinary Laboratories Agency, United Kingdom

Disease surveillance has been defined in many ways, all incorporating the following main elements: the ongoing collection, validation, analysis and interpretation of health and disease data that are needed to inform key stakeholders and allow them to take action by planning and implementing more effective, evidence based policies and strategies relevant to the prevention and control of disease.

The importance of pathology in new and emerging animal disease detection and characterisation has been well recognised, for example BSE, WNV99, PCVAD and bovine neonatal pancytopenia. Pathosurveillance denotes the role of pathologists in scanning surveillance for new and emerging animal diseases, which involves generation and analysis of observational data, formulation of hypotheses on possible causation and devising approaches to test these.

With the increasing emphasis on early threat detection there is a need to extend and promote this aspect of veterinary pathology. The European Pathosurveillance Network (EPSN) was established in 2009 to provide a communications network for veterinary pathologists working in farm animal diagnostic investigation. The aims of the EPSN are to promote early recognition of emerging diseases in Europe and support rapid knowledge transfer between pathologists, including updates on new and emerging / re-emerging diseases, sharing methodologies and best practices and collaboration on projects on new diseases or diseases of unknown aetiology. Future challenges include harmonisation of nomenclature to improve data exchange and promoting the role of pathologists in disease surveillance.

Invited Talk

I2: TOP TIPS FOR PUBLISHING A RESEARCH PAPER IN THE JOURNAL OF COMPARATIVE PATHOLOGY

Michael J. Day

School of Veterinary Sciences, University of Bristol, United Kingdom

The Journal of Comparative Pathology is one of two international journals in which original research in veterinary pathology may be published. The Journal was founded in 1888 by Sir John M'Fadyean and recently celebrated its 125th anniversary with the launch of a complete on-line archive from inception to the current day. Access to and information about the Journal and the associated Journal of Comparative Pathology Educational Trust is via the Journal's website at: http://www.journals.elsevier.com/journal-of-comparative-pathology/. The Journal is published by Elsevier and has a current impact factor of 1.376 with a 5-year impact factor of 1.675. The Journal is published monthly as eight issues per year in two volumes (four issues are double issues).

The Journal publishes high quality articles on all aspects of the pathology of diseases of domesticated and other vertebrate animals. Topics covered include histopathology, ultrastructure, microbiology, immunology, toxicology, parasitology, and functional, molecular and clinical pathology. Articles on diseases of man are also included if they present features of special interest when viewed against the general background of vertebrate pathology.

The Journal publishes original research papers, review articles and short papers (case studies) under five subsections: (1) infectious disease, (2) neoplastic disease, (3) spontaneously arising disease, (4) experimentally induced disease, and (5) disease in wildlife or exotic species. This presentation addresses the publication of original research papers. Needless to say, such submissions must contain novel and previously unpublished research data that make a significant contribution to scientific knowledge.

Complete information regarding the requirements and format for an original research paper are found in the Notes for Contributors that are published on-line and in occasional issues of the Journal. Authors must consult these guidelines and are also advised to examine a recent copy of the Journal to ensure correct format is followed.

Original research papers are divided into five distinct subsections: (1) Summary, (2) Introduction, (3) Materials and Methods, (4) Results, and (5) Discussion. The authorship of papers should be considered carefully. The Journal does not yet ask for details of the contribution of each author, but each author listed should have made a distinct and quantifiable contribution to the work described.

The Summary (note the Journal does not use the term 'Abstract') is best written last. It should be concise and describe the main findings of the work, indicating their significance against the background of current knowledge. A list of no more than four key words should follow the Summary.

The Introduction should review the published literature in the field and conclude with a clear statement of the aims of the current research against that background.

The Materials and Methods should be presented in subsections, generally in chronological order of the work undertaken. Typically, this might entail sections devoted to: (1) Case material or experimental animal source, housing, grouping and procedures, (2) gross pathological examination, (3) histopathological examination, (4) other testing (e.g. immunohistochemistry, microbiology, molecular testing), and (5) statistical analysis. For the latter, the statistical tests, the level of significance and the computer programmes utilised should be given. In experimental studies it is essential to provide information about the ethical review process that has been undertaken.

Results should be presented in subsections that follow those used in the Materials and Methods and follow the same chronological order. Careful consideration should be given to which data are presented as text, tables, graphs or photomicrographs. The key is not to duplicate information within these different formats. Tables and figures must NOT be embedded in the text of the document, but submitted separately. The precise format for tables should be followed.

Tables must be drawn using the 'table' function of the word programme and NOT submitted in excel or other formats. Photomicrographs must be submitted as individual high-resolution jpeg or tiff files. Photomicrographs should be clear and focused with optimum contrast and balance and be taken of sections with excellent staining quality. Extraneous detail should be cropped. Photomicrographs should include a size bar (with size indicated in the figure legend if not associated directly with the bar) and any relevant arrows or arrowheads (that should be of sufficient size that they remain visible if figures are reduced in size for printing). Figures should NOT be submitted as PowerPoint files of composite images. Figure layout will be determined by the Journal page setters. Although supplementary material may be submitted for on-line access, careful consideration should be given as to whether this is essential.

The Discussion should review the results of the study in light of the current knowledge in the field. The discussion should be as concise as possible and end with a concluding statement (there is no separate 'Conclusions' section).

The Discussion should be followed immediately by any Acknowledgments and then by a separate Conflict of Interest Statement. These statements are in turn followed by the list of References. It is crucial to follow the Journal's referencing format precisely as laid out in the Notes for Contributors. Incorrect referencing format adds considerably to the time taken for manuscripts to be edited and slows the publication process. The list of Figure Legends should follow the References.

Authors whose first language is not English are strongly advised to have their manuscripts read by a native English-speaking colleague or to use a professional manuscript editing service. Manuscripts of poor English standard will not be sent for peer review. Note that the Journal uses British English and any spell-check programme should be set to that format.

Manuscripts, figures and tables should be submitted separately with a covering letter by uploading the files to the Elsevier Editorial System (EES) used by the Journal. Once authors have registered with the system it will lead you through the stages of on-line submission.

Research papers are sent to two independent referees for peer review. Once these reviewer's reports are submitted a final decision will be made by the Editor-in-Chief. A manuscript will either be rejected (with no further communication entered into) or returned for revision, which may entail further review. It is very rare for a paper to be accepted without some level of revision. Revised manuscripts are again uploaded to EES after which a final decision will be made on acceptance. Acceptable papers will then be edited for style, grammatical correctness and format and returned to the communicating author for final approval. Only after this stage will papers be formally accepted for publication. Papers will then be page-set and proofs will be returned to the authors in addition to being scrutinised by the Journal's proof reader and the Editor-in-Chief. Final page proofs will then become available on-line with a citable DOI number. Manuscripts are published in hard copies of the Journal in date order of acceptance.

Invited Talk

I3: SECRETS OF THE JOURNAL REVEALED: AN ANALYSIS OF EDITORIAL DECISIONS FROM THE EDITORS OF *VETERINARY PATHOLOGY*

Andrea Gröne and Jeff L. Caswell

Department of Pathobiology, Faculty of Veterinary Medicine, Utrecht University, the Netherlands (A. G.)

Department of Pathobiology, University of Guelph, Canada (J. L. C.)

Pathologists who submit their work to scientific journals have invested much effort and cost by the time the manuscript arrives in the editorial office. The peer review model of publishing scientific papers has strong support, yet authors may perceive the process in a more complex way: hypercritical, painful, combative, and quibbling, yet necessary, valuable, revealing, insightful, thought-provoking and important for quality assurance. And it is shrouded in secrecy: the confidentiality accorded to authors and reviewers can sometimes obscure the very nature of the editorial process. What happens to my manuscript when it enters this monstrous editorial machine?

This seminar begins by demystifying the process by which decisions are made by the editorial staff of Veterinary Pathology. Editorial decisions are based on the quality and novelty of the work, the potential value for readers, appropriateness of the work to the scope of the journal, insights and analysis provided by peer reviewers and by the editorial staff, and authors' responses to these criticisms. We suggest approaches to creating a rebuttal letter that appropriately address the critical comments of reviewers and editors.

Included is an informal analysis of the most recent 200 submissions to the journal, as an attempt to illustrate the editorial process, the reasons that manuscripts are accepted or rejected, and how they are modified along the way. Analysis of accepted manuscripts reflects the defined scope of the journal, as well as an expectation that manuscripts must (1) have significant importance to animal and/or human health, 2) address disease mechanisms/pathogenesis and/or clinico-pathologic correlations and/or descriptions of important new or emerging diseases, 3) include new knowledge supported by valid data, and 4) be of sufficiently broad interest to be relevant to veterinary pathologists. Conversely, there is often more than one reason when manuscripts are rejected, and we categorise these relative to the scope of the journal, the novelty and value of the findings, whether the findings advance knowledge, the magnitude of the study and sufficiency of the data, considerations of methodology and study design, specific requirements of Case Reports and Diagnostic Exercises, and quality of presentation of the work.

The intent of the seminar is constructive, for participants to apply this information in a critical analysis of their own work. From the earliest inception of the study to the final approval of manuscript proofs, authors make important decisions about study objectives or hypotheses, methods and experimental design, validation of results, follow-up or confirmatory investigations, interpretation of findings, organisation of the findings, modes of presentation, clarity of writing, revision and more revision, quality of images, and responses to criticisms. We will provide insights on our experiences with manuscripts submitted to Veterinary Pathology, with the hope that participants may find this information of value in successfully conducting and publishing their own investigations and research.

Invited Talk

I4: THE INTERNATIONAL SOCIETY OF VETERINARY DERMATOPATHOLOGY: OBJECTIVE, GOALS AND FUTURE PROSPECTS

Laura Bongiovanni

Faculty of Veterinary Medicine, University of Teramo, Italy

The love for the study of veterinary and comparative skin diseases, the enthusiasm for teaching, together with the willingness to connect individuals who have the same professional interest and develop veterinarians who are interested in the histologic interpretation of skin diseases, led to the creation of a specialised organisation: the International Society of Veterinary Dermatopathology (ISVD).

Founded in 1998, the ISVD has today over 160 members, belonging to more than 23 different World Countries, with the advancement of veterinary and comparative dermatopathology as its major goal.

The ISVD aims to encourage emerging technologies for the diagnosis of skin diseases in animals, to increase the understanding of veterinary dermatopathology and to provide the opportunity for graduate veterinarians to learn advanced techniques and new concepts in the field.

The society reaches these aims through providing meetings, seminars, and courses which provide the opportunity for graduate veterinarians to learn advanced techniques and emerging concepts in veterinary dermatopathology.

The international nature of the ISVD is highlighted by its cosmopolitan community, comprising both pathologists and dermatologists, the interest in skin diseases all over the world, and by the commitment to organise rotating ISVD annual meetings between the east and west hemispheres and, every four years, a joined meeting with the World Congress of Veterinary Dermatology.

In the future, the ISVD intends to further its goals of promoting dermatopathology via the current method of hands on workshops but also by embracing on-line teaching and supporting both the European and American Societies of Pathology and Dermatology in their resident training.

Workshop

W1: LESION SCORING AND DIGITAL IMAGE ANALYSIS

Robert Klopfleisch

Institute for Veterinary Pathology, Freie Universität Berlin, Germany

Histopathology was first used and is still used today to diagnose infectious, degenerative or neoplastic diseases in humans or animals. These qualitative diagnoses are based on a sum of observable changes in the morphology of the analysed tissue. The cognition of these changes is based on the pattern recognition of the observer and the comparison of these patterns with the known physiologic variation in tissue morphology in the respective species. Decades of experience in veterinary pathology show that this approach allows for reproducible qualitative diagnoses by the observer but can also be used for semi-quantitative scoring of the lesions magnitude, i.e. on an ordinal scale for instance with a low, medium or high grade trichotomy which correlates with the clinical relevance of the lesions (1).

Absolute quantification of the lesions extent and severity is however difficult since two main problems hamper absolute quantification, i.e. on a rational scale with absolute values of 1, 2, 3 etc., using standard, non-automated histopathology. First, the detection method is not reliable enough. Despite intensive training and attempts to standardise nomenclature and the definition of lesions there are still unresolved issues in terms of inter-observer variation which may be acceptable for qualitative and semi-quantitative evaluation but not for absolute quantitation (2). Second, in most circumstances it is impossible to objectively justify the interval between two values, thus a read out of histopathologic scoring on a rational scale is difficult. Image analysis by automated calculation of the tissue area affected or cells present per area have been introduced to overcome this problem. These approaches aim at a reliable and reproducible histopathology read out in a rational scale to allow proper statistical processing and at an exclusion of an observer bias [3,4]. Image analysis approaches usually use one or only few two dimensional planar sections of the tissue of interest to measure three-dimensional objects. This two-dimensional approach thus may also lead to biased results. Stereology, which is based on systematic random sampling and estimates third dimensional information, has been developed to avoid this bias [3]. It can therefore be seen as the most sophisticated method for the quantification of histologic information. It is however a laborious and complex method which is established in only few laboratories. Semi-quantitative scoring systems are therefore still the mostly used methods to include histopathologic information in biomedical research. These scoring systems usually include multiple parameters which are separately quantified on an ordinal scale and finally combined in a total score. Average scores of the different experimental groups can then be compared by non-parametric statistical tests. The selection of the parameters should be based on the scientific hypothesis or question together with the current knowledge on the morphologic outcome of the investigated disease model. It may therefore be useful to design an individual scoring system for each study which in the best possible way answers the particular scientific question. Standard scoring systems for specific disease models on the other hand allow for the comparison of the results of different studies. Several standard scoring systems for different mouse models have been introduced or emerged in the past 20 years. Histopathologists are therefore repeatedly requested by cooperating scientists to evaluate the outcome of animal studies using standard scoring systems or to elaborate project specific scoring systems.

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Workshop

W5: GENOMICS AND PROTEOMICS IN VETERINARY PATHOLOGY

Robert Klopfleisch

Institute for Veterinary Pathology, Freie Universität Berlin, Germany

For decades, research on genetic diseases and especially oncology has been driven by the search for "the one" mutation or at least "the one" mechanism that is mainly initiating and forcing disease development. This desire for a uni- or at least oligodimensional molecular pathogenesis is supposedly caused by the tendency of human cognition to reduce complexity for better understanding but also by the pragmatic wish to find a simple therapeutic approach to all patients with the clinically defined disease. However the results of cancer research in the first decade of this century dashed the hope for a simple answer to our questions on tumor carcinogenesis. First and foremost the introduction and exponential improvement of the so called -omics technologies answered many questions regarding tumor biology. One of the major findings of these technologies was the identification of a tremendous variability of tumor genomes, epigenomes and proteomes. Tumors which were regarded as clinically and morphologically identical and therefore treated similarly suddenly became less related than previously thought.

Genomic analysis was restricted to short sequences or few genes before the introduction of high-throughput genomics- and proteomics technologies like microarray analysis and next generation sequencing (NGS). The introduction of the latter helped tremendously in the understanding of disease associated genomes and transcriptomes and therefore helped to cross so far well established borders and allowed for leaps in knowledge and new paradigms. Microarray technology was the pioneer technology to analyse numerous gene fragments in parallel thus allowing the analysis of almost the complete genome and transcriptome in one analysis without a specific hypothesis. This analysis was however restricted to known nucleotide sequences which were placed onto the array. The potential read outs were thus the presence or absence or the quantity of a known nucleotide sequence in a tissue lysate.

In recent years NGS is increasingly replacing microarray analysis, since it has several major advantages over microarrays. First, NGS includes a de novo sequencing of each genome and thus allows for hypothesis-independent detection of so far unknown sequences, i.e. single nucleotide polymorphisms or larger mutations in addition to known nucleotide sequences.

One of the problems associated with the advantage of massive parallel sequencing is the sequencing inaccuracy due to the vast amount of sequences analysed and the difficult differentiation of germline polymorphisms/mutations from actual mutations. These problems can nevertheless be solved by increasingly improved software and sequencing of paired tissue samples of diseased and non-diseased tissues of the same individual. Indels or copy number variants and thus very small or very large insertions and deletions are however still difficult to be identified (2). Despite these problems, NGS has proved a tremendous aid to the understanding of the genomic nature of several human diseases, e.g. inter- and intratumoral variability in the genomes in tumor groups which have been regarded as being homogenous before. Besides or maybe because of its impact on basic oncology research, NGS is also regarded as close to being used in daily diagnostic routine. The slowly but constantly decreasing costs of NGS bring sequencing of the exome closer from bench to bedside. The usefulness and acceptance of these diagnostic tests are however very much dependent on the influence of their results on the therapeutic success, that is the availability of drugs targeting the pathways changed by the identified mutations.

Despite the similar impressive progress in the development of proteome analysis methods like chromatography and mass spectrometry their contribution to the identification of malignancy- and therapy response-predicting protein patterns is much smaller. Proteome analysis in general aims for the same results as NGS. However, due to the high chemical variability of proteins in the proteome a complete overview on the presence and changes in the structure of proteins is impossible. This problem can nevertheless be avoided by pre-fractionating of the complex tissue, for instance purification of the phosphoproteins. In terms of biomedical research proteomic methods are mostly used for the identification of

biomarkers in clinically accessible body fluids such as plasma or serum. These fluids are however problematic due to their high abundance of serum albumin in plasma or the loss of albumin-bound proteins after albumin removal. Initially, proteome analysis was mainly based on two-dimensional gel electrophoresis and subsequent mass spectrometry (MS). Due to the problematic intergel variability the more robust liquid chromatography separation coupled with MS is increasingly used. In this approach proteins in the analysed samples are digested and separated by size or other inherent parameters to reduce complexity of the sample before identification of the peptide sequence by MS. There is also an increasing effort to establish antibody libraries that recognises all protein components of the human proteome. These antibodies could be used in high throughput tissue microarrays (TMA) to analyse the presence and concentration of a protein in situ therefore adding the information of spatial distribution of a protein in a complex tumor.

Taken together, "omics"-technologies have tremendously increased the knowledge base on the pathogenesis of several diseases. This information is now increasingly used to tailor therapeutic approaches towards diseases and to increase the efficiency and efficacy of the treatment efforts.

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Trainee Workshop

TW2: COACHING IN WRITING AND PUBLISHING A RESEARCH PAPER IN THE *JOURNAL OF COMPARATIVE PATHOLOGY*

Michael J. Day

School of Veterinary Sciences, University of Bristol, United Kingdom

For detailed information see page 49–50.

Mystery Slides in Veterinary Dermatopathology ESVP/ECVP

OLIVIA: LOCALISED PAPULO-CRUSTOUS DERMATITIS IN A RABBIT

***L. Bongiovanni*[*], *N. Di Girolamo*[†], *P. Selleri*[†]**

[*]*Faculty of Veterinary Medicine – University of Teramo, Italy and* [†]*Private Practitioner Centro Veterinario Specialistico, Clinica per Animali Esotici - Rome, Italy. e-mail: lbongiovanni@unite.it*

Signalment: Rabbit, mixed breed lop rabbit, female (intact), age unknown

History: The medical history of the patient was unknown. Four days before the visit the owner observed a swelling affecting the dorsal aspect of the nose.

Clinical/gross findings: The animal was in good nutritional condition and two skin lesions were evident: a papular lesion affecting the dorsal aspect of the nose (biopsy) and a 15-mm, crusting, ulcerative lesion located on the cranial margin of the left ear pinna.

Sample: Haired skin, biopsy, FFPE, HE-staining

Label: ISVD -CASE 1

Your diagnosis / notes:

BOOFI: A CASE OF EXFOLIATIVE DERMATITIS WITH A POSSIBLE UNUSUAL AETIOLOGY?

S. Bettenay

Tierdermatologie Deisenhofen, Germany. e-mail: s-bettena@t-online.de

Signalment: Dog, Elo, female (intact), 9 years

History: The patient was presented with a 6 week history of pruritus and widespread inflammatory skin lesions. In addition the dog had polyuria/polydipsia and reported weight loss. Punch biopsies were performed (slide A). There was a slow and incomplete response to antibiotic therapy. After 6 months, the skin was re-biopsied due to the incomplete response to therapy (slide B).

Treatment: Pradofloxacin oral

Gross findings: Skin lesions ranged from hypotrichosis to alopecia and scaling to exfoliation with associated erythema.

Sample: Skin biopsy (A), skin re-biopsy (B), FFPE, HE-staining

Label: ISVD -CASE 2

Your diagnosis / notes:

Mystery Slides in Veterinary Dermatopathology ESVP/ECVP

MEMPHIS BELLE: PROGRESSIVE SKIN LESIONS ON FACE, HEAD, PINNAE AND TRUNK IN A DOG

V. K. Affolter

William Pritchard Veterinary Teaching Hospital, Department of Pathology, Microbiology, immunology, UC Davis, USA. e-mail: vkaffolter@ucdavis.edu

Signalment: Dog, smooth coated collie, female (spayed), 8 years

History/gross/clinical findings: The patient was presented with papules, small plaques and crusts mostly on the face and pinnae. Lesions developed over a period of a few weeks and were not pruritic. The lesions were not responsive to antibiotics.

Treatment: Antibiotics

Sample: Skin, biopsy, FFPE, HE-staining

Label: ISVD -CASE 3

Your diagnosis / notes:

ISOTTA: SCALING DERMATITIS WITH ERYTHEMA AND PRURITUS IN A DOG

***C. Brachelente*[*], *A. Fondati*[†]**

[*]Department of Veterinary Medicine – University of Perugia, Italy and [†]Private Practitioner Centro Veterinario Prati – Rome, Italy. e-mail: chiara.brachelente@unipg.it

Signalment: Dog, Dachshund, female (spayed), 14 years

History/clinical findings: The dog had suffered from recurrent otitis and pruritic dermatitis for more than 6 years. In the last 8-9 months, the dog had been suffering from progressive weight loss with a poor quality haircoat, generalised scaling and erythema with alternating pruritus. There was a poor response to treatment with prednisolone (1 mg/Kg po die). Two punch biopsies were taken.

Treatment: Prednisolone (1 mg/Kg orally, once a day)

Gross findings: Generalised scaling and erythema

Sample: 2 x haired skin, biopsy, FFPE, HE-staining

Label: ISVD -CASE 4

Your diagnosis / notes:

Mystery Slides in Veterinary Dermatopathology ESVP/ECVP

TIGRE: PROGRESSIVE SWELLING OF THE RIGHT FRONT LEG IN A CAT

P. Roccabianca

DIVET-University of Milano, Italy. e-mail: paola.roccabianca@unimi.it

Signalment: Cat, domestic short hair, male (neutered), 10 years

History: The patient developed slow progressive swelling followed by erosions and intermittent multifocal bleeding of the distal right (mostly dorso-lateral) forepaw extending progressively from carpus to the forearm. Occasional chewing was reported. On palpation the paw was non painful and cold. Systemic signs were not present. There was no response to antibiotic therapy (clindamycin 10mg/kg/sid). Four punch biopsies were performed (slide A). Due to progressive worsening and after the first biopsy results the leg was amputated (slide B).

Treatment: Clindamycin 10mg/kg once a day for 14 days

Clinical/gross findings: Slow progressive swelling, erythema, erosions and intermittent bleeding of the distal right (mostly dorso-lateral) paw extending progressively to the carpus and forearm

Sample: Skin, biopsy (A- pre-amputation), Skin (B post-amputation), FFPE, HE-staining

Label: ISVD -CASE 5

Your diagnosis / notes:

AIDEN: ERYTHEMATOUS, EROSIVE LESIONS ON THE MUZZLE IN A DOG

V. K. Affolter

William Pritchard Veterinary Teaching Hospital, Department of Pathology, Microbiology, immunology, UC Davis, USA. e-mail: vkaffolter@ucdavis.edu

Signalment: Dog, shepherd mix, male (castrated), 2 years

History: The patient developed scaly lesions on the muzzle and nasal planum. Within a few days erythema, erosions and crusts were noted and the dog was presented to the VMTH.

Treatment: Antibiotics after collection of biopsies

Gross/clinical findings: The dog showed scaling and erythema with very small crusts on the nasal planum and muzzle.

Sample: Haired skin, biopsy, FFPE, HE-staining

Label: ISVD -CASE 6 (back-up)

Your diagnosis / notes:

INDIVIDUAL EQUIPMENT FOR VETERINARY PATHOLOGIES

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Oral Presentations ESVP/ECVP

Oral Presentation Schedule

Oral Session	Time	Room	Starts at	Session Leader	Abstract numbers
EPSN-European Pathosurveillance Network	Thu, 11:00–12:45	A/B	EPSN01	S. Scholes, N. Borel	EPSN01-EPSN07
1 Reproduction	Thu, 12:00–12:45	C	O01	E. Karlstam, J. Polona	O01–O03
2a Oncology I: Mammary Cancer	Thu, 2:15–4:00	A/B	O04	J. Abadie, S. Rottenberg	O04–O10
2b Spontaneous and Experimental Neuropathology	Thu, 2:15–4:00	C	O17	A. Oevermann, T. J. Rosol	O17–O23
3a Oncology II: Spontaneous and Experimental Oncology	Thu, 4:30–6:00	A/B	O11	L. Peña, C. Brayton	O11–O16
3b Toxicology	Thu, 4:30–6:00	C	O24	J. Caswell, C. Botteron	O24–O29
4 Emerging and Other Infectious Diseases of Current Interest	Fri, 8:15–10:00	C	O30	M. Aleksandersen, W. Baumgärtner	O30–O36
5 Zoo and Wild Life Diseases	Fri, 10:30–12:00	C	O37	A. Kipar, A. Sukura	O37–O42
6a Host Pathogen Interactions	Fri, 1:45–3:15	A/B	O43	J. McKay, M. Day	O43–O48
6b Outstanding Case Reports	Fri, 1:45–3:15	C	O49	P. Roccabianca, J. Szarek	O49–O54
7a Nanotoxicopathology	Fri, 5:15–5:45	A/B	O55	P. Germann, F. Schorsch	O55–O56
7b Methodological Developments	Fri, 5:15–5:45	C	O57	A. Gröne, K. Chiers	O57–O58

Oral Abstract Register

European Pathosurveillance Network (EPSN) Oral Presentations

EPSN01 PATHOSURVEILLANCE – PAST, PRESENT AND FUTURE

S. Scholes

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Disease surveillance has been defined in many ways, all incorporating the following main elements: the ongoing collection, validation, analysis and interpretation of health and disease data that are needed to inform key stakeholders and allow them to take action by planning and implementing more effective, evidence based policies and strategies relevant to the prevention and control of disease.

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With the increasing emphasis on early threat detection there is a need to extend and promote this aspect of veterinary pathology. The European Pathosurveillance Network (EPSN) was established in 2009 to provide a communications network for veterinary pathologists working in farm animal diagnostic investigation. The aims of the EPSN are to promote early recognition of emerging diseases in Europe and support rapid knowledge transfer between pathologists, including updates on new and emerging / re-emerging diseases, sharing methodologies and best practices and collaboration on projects on new diseases or diseases of unknown aetiology. Future challenges include harmonisation of nomenclature to improve data exchange and promoting the role of pathologists in disease surveillance.

Notes:

EPSN02 EPIZOOTIC INFECTION OF A SWISS SWINE HERD WITH *PORCINE CYTOMEGALOVIRUS*

***H. Marti*[*], *A. Stahel*[†], *K. Hoffmann*[*], *K. Wäsle*[*], *M. Engels*[†], *T. Sydler*[*], *E. Bürgi*[‡] *and M. Hilbe*[*]**

[*]Institute of Veterinary Pathology and [†]Veterinary Virology, and [‡]Department of Farm Animals, University of Zurich, Switzerland. hanna.marti@uzh.ch

Introduction: *Porcine cytomegalovirus* (PCMV, *suid herpesvirus 2)* is a beta herpesvirus. Orr et al. (1988) reported a disease outbreak in a minimal disease swine herd experiencing high mortality and reproductive losses most likely caused by PCMV, though isolation of the virus was not possible.

Materials and Methods: Within the course of 19 days (late October to early November 2013), necropsy examinations were performed on twenty-one pigs from one Swiss herd. These included thirteen piglets from the age of 1 to 4 weeks (average: 2.2 weeks), one eighteen-week-old pig, one sow, and, from a single litter, one stillbirth with five foetuses and one piglet. The general clinical history featured weakness in the piglets and poor growth. Several pigs showed lameness (n=4), respiratory symptoms (n=3) and fever (n=2). The pigs were evaluated by histopathology (n=21) and molecular tests for herpesvirus (n=9) and adenovirus (n=3).

Results: Histologically, intranuclear inclusions were found in various organs of eighteen pigs: spleen (n=8), nasal mucosa (n=7), liver (n=3), lung (n=1) and mammary gland (n=1). Morphologically, the inclusions in the nasal mucosa and the mammary gland were indicative for PCMV. In five piglets, there was indication for a systemic herpesvirus infection and secondary septicaemia with moderate purulent splenitis (n=5), hepatitis (n=4), mild mixed cellular meningoencephalitis (n=4) and mild interstitial pneumonia (n=2). By PCR, all examined pigs were positive for herpesvirus and negative for adenovirus. Sequencing of the amplified PCR product revealed cytomegalovirus.

Discussion: This case study reports an epizootic outbreak of cytomegalovirus in a swine herd in Switzerland.

Notes:

European Pathosurveillance Network (EPSN) Oral Presentations

EPSN03 INHERITED α-MANNOSIDOSIS IN CROSSBREED CALVES

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Introduction: A wide variety of lysosomal storage diseases has been reported in humans and domestic animals. In cattle has been reported in Aberdeen Angus, Murray Grey and Galloway. We describe an outbreak of inherited α-mannosidosis in inbreeding crossbreed calves.

Materials and Methods: During the breeding period 2007-2008, in a herd of 1 bull Bradford/Limousin and 80 crossbred cows (mainly Hereford, Aberdeen Angus), 10 out of 20 daughter cows were bred by their father. Then, 6 out of 10 calves developed severe neurological signs from 2-3 months old, dying 2 months later. Clinical signs were ataxia, incoordination, standing difficulty, seizures, circling movements, anorexia and death. Necropsy was performed on two calves, no gross lesions were detected. Samples of CNS and spleen were fixed in formalin 10%, for histopathology (HE stain), lectinhistochemistry (LHQ) with 9 different biotinylated lectins (Con-A, SBA, DBA, UEA-I, WGA, s-WGA, PNA, RCA-I, BS-I), or in glutaraldehide 2.5% for ultrastructural observation. To determine the mutation (T961→C transition), 31 blood samples (from the bull and 30 cows), and hair samples from 6 calves with neurological signs were taken.

Results: Multiple cytoplasm vacuoles in different tissues were the main lesion; cytoplasmic vacuolation was particularly evident in neurons. The stored material reacted strongly with Con-A, WGA, and sWGA. The ultrastructural examination showed lysosomal stored material characterised by heterogeneous electron-dense membranous bodies and empty vacuoles in the perikaryon of affected neurons and in axonal spheroids. Molecular analysis demonstrates the presence of the T961→C transition.

Conclusion: We confirmed inherited α-mannosidosis in crossbreed affected cattle.

Notes:

EPSN04 DETECTION OF POLYTREPONEMAL INFECTION IN THREE CASES OF PORCINE ULCERATIVE STOMATITIS BY FLUORESCENT IN SITU HYBRIDISATION

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Introduction: Ulcerative and fibrino-necrotising stomatitis is occasionally reported in pigs. The aetiology is unknown but spirochetes are observed in silver stained sections, however, the spirochetes have never been identified. The aim of this study was to report three cases of fibrino-necrotising stomatitis associated with polytreponemal infection.

Materials and Methods: Tissue samples from the oral cavity of three sows euthanised due to ulcerative stomatitis, ulcerative vaginitis and Mortellaro-like alterations of the feet were fixed in formalin, paraffin embedded and sectioned for H&E and serial fluorescent in situ hybridisation (FISH) for identification of spirochetes with genus and species specific oligonucleotide probes targeting 16S or 23S ribosomal RNA. The presence of *Fusobacterium necrophorum* and *Domain Bacteria* was additionally detected by FISH.

Results: Diffuse fibrino-necrotising infection affecting the epidermis and dermis of the entire oral cavity was found in all sows. By FISH all lesions showed severe bacterial infiltration with a strong predominance of spirochetes. Additional hybridisation revealed that the spirochetes all belonged to genus *Treponema*. Neither, *Brachyspira*, *Borrelia*, nor *Leptospira* organisms were detected. Further identification of the spirochetes showed intermingled infection with *Treponema pedis*, *Treponema putidum* and up to eight other *Treponema* species/phylotypes. Low numbers of *F. necrophorum* was also detected but only the treponemes were infiltrating deep into the lesions to the borderline between damaged and vital tissue.

Discussion: Using a panel of genus specific probes this is the first study identifying the involvement of *Treponema spp*. in porcine ulcerative stomatitis. All three sows revealed polytreponemal infections with *Treponema* species/phylotypes commonly associated with bovine digital dermatitis and human periodontal disease.

Notes:

European Pathosurveillance Network (EPSN) Oral Presentations

EPSN05 LESION PATTERNS ARE RELATED TO THE QUANTITY OF VIRUS AND SEROLOGICAL RESPONSE IN OVINE VISNA/MAEDI

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Introduction: Different lesion patterns have been described in neurological forms of ovine Visna/Maedi (VM) related to individual inmunopathological response. In this work we describe the main lesion patterns observed in lung, central nervous system (CNS) and mammary gland and their association with the VM virus antigen present in tissue and the serological response.

Materials and Methods: Lung, CNS and mammary gland sections from 34 natural clinical cases of VM in adult sheep were examined. Inmunohistochemistry was used for the detection of VM virus antigen and an ELISA test was carried out to evaluate serological response.

Results: Sheep showed lymphocytic (n=10), histiocytic (n=19) or intermediate (n=5) lesion patterns. Lymphocytic lesions were characterised by $CD4^+$/$CD8^+$ T cells predominance while in histiocytic lesions macrophages and B cells were the predominant cells. All showed MV lesions in lung, 32 in CNS and 26/29 in mammary gland with different intensity degrees in each tissue. Severe lesions were only located mainly in one organ. All animals were positive for MV antigen, being higher in the histiocytic pattern. A high serological response was observed in ELISA test in the histiocytic pattern, being much lower or even negative in the lymphocytic type.

Discussion and Conclusions: In this study an association between lesion patterns, presence of VM virus antigen in tissue and serological response has been confirmed and related to the individual response. The features of the individual inmunopathological response, including the presence of seronegative animals, should be considered in the diagnosis and control of MV.

Notes:

EPSN06 IMMUNOHISTOCHEMICAL, MORPHOMETRIC AND HISTOPATHOLOGICAL STUDY OF THE THYMUS OF CALVES WITH SUBCLINICAL BVD AND HEALTHY CALVES, BOTH CHALLENGED WITH BHV-1

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Introduction: Since the thymus is a target organ for the *bovine viral diarrhoea virus* (BVDV), our experiment aimed to understand its relationship with the immunosuppressive effect, by studying the consequences of a previous infection with BVDV on the thymus of calves challenged with BHV-1.

Materials and Methods: 24 Friesian calves were used in this study: 12 animals were inoculated intranasally with a non-cytopathic BVDV-1 strain 7443; 12 days later, 10 of them were coinfected intranasally with *bovine herpesvirus 1.1* (BHV-1) strain Iowa. These animals were euthanised in batches of two between 0 and 14 dpi with BHV-1. Another 10 calves were inoculated solely with BHV-1 and euthanised in batches of two between 0 and 14 dpi with BHV-1. Thymus samples were processed for histopathological and immunohistochemical studies focused on BVDV/BHV-1 antigens, collagen (Masson's trichrome), Factor VIII, corticomedullary ratio and level of apoptosis.

Results: A complete absence of BHV antigen in both groups and the presence of BVD antigen in the coinfected group were observed. BVDV-preinfected animals showed a thymic cortex depletion associated with a reduced ratio cortex: medulla, and a higher presence of tingible body macrophages, collagen and vascular structures. Calves solely infected with BHV-1, despite having the same age, did not show degenerative changes until the end of the experiment.

Discussion: These findings could affect not only the numbers of circulating and local mature T cells but also the T cell-mediated immunity, which seems to be impaired during infections with this virus, thus favouring pathogenic effects during secondary infections.

Notes:

European Pathosurveillance Network (EPSN) Oral Presentations

EPSN07 *INFECTIOUS BRONCHITIS VIRUS* PATHOGENESIS: THE NASAL MUCOSA IS AN IMPORTANT REPLICATION SITE OF IBV M41 DURING EARLY INFECTION

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Introduction: Infectious bronchitis in chickens is a highly variable respiratory and urogenital disease, caused by a wide range of subtypes of the *Infectious Bronchitis Virus* (IBV) (family *Coronaviridae*). To effectively deal with this disease in poultry industry in the future (either therapeutically or protective), detailed knowledge about the pathogenesis is needed. Since not much is known about the early stages of infection, our study investigates viral distribution in the host and host reactions with emphasis on this initial period.

Materials and Methods: 42 chickens (SPF layer, 7 days old) were intranasally inoculated with 1 x 10^5 EID of IBV type Massachusetts 41. At 6, 12, 24, 36, 48, 72 and 144 hours post inoculation (hPI), 6 inoculated and 2 mock-inoculated animals were euthanised and 32 tissues were sampled for histological and immunohistochemical (anti-IBV) analysis and quantitative PCR.

Results: Histologically, moderate to severe epithelial degeneration, attenuation, desquamation and deciliation with loss of submucosal glands and intraepithelial and submucosal lymphohistiocytic and heterophilic infiltration were found in the nasal mucosa and trachea, starting at 24 hPI. Immunohistochemical staining showed expression of viral proteins in mainly ciliated epithelium of the nasal mucosa in most of the animals between 12 and 72 hPI and to lesser extent in the trachea and conjunctiva.

Discussion (and/or Conclusions): Our data suggest that the nasal mucosa might be an important replication site for IBV M41 during early infection. Further analysis by qPCR and immunohistochemistry will allow confirmation and quantification of identified and localised viral presence in these and other tissues.

Notes:

Oral Presentations ESVP/ECVP

O01 THE PROGNOSTIC VALUE OF FUNCTIONAL DISORDERS IN THE EQUINE ENDOMETRIUM WITH REGARD TO THE TIME OF DIAGNOSTIC BIOPSY SAMPLING

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Introduction: The prognostic relevance for successful breeding of endometrial maldifferentiation in spring and autumn is uncertain in mares. The aim of this study was to outline an annual time span for effective prognostic biopsy sampling by means of a morphofunctional characterisation of the endometrium during the spring and autumn transitional period (STP/ATP).

Materials and Methods: Endometrial specimens from mares with detailed clinical documentation were taken in February, March and April to outline the STP (n=187). Samples taken in September, October and November were chosen to investigate the ATP (n=77). All biopsies were examined microscopically with emphasis on the functional morphology of the endometrium (activity, glandular and stromal differentiation, immunohistochemistry [hormone receptors, Ki67-antigen, selected specimens]).

Results: Until early April (STP), the endometrial activity was predominantly absent or low, whereas during ATP no or only low activity was ascertained in more than 60% of all specimens from late October onwards. About 27% of all evaluated specimens (summarising STP and ATP) revealed differentiation disorders, mainly classified as 'irregular' and to a lesser amount as 'unequal'. A comparison of the endometrial functional morphology with the ovarian status (evaluated clinically) showed an accordance of these findings in about 60 to 81% and nearly 60 to almost 70% of all mares examined during STP and ATP, respectively. The immunohistochemical evaluation was supportive of the histomorphological findings.

Discussion (and/or Conclusions): Endometrial maldifferentiations are frequently found in biopsies taken during the transitional periods. Therefore, diagnostic biopsy sampling should be performed from May onwards and before September.

Notes:

O02 FROM MONO TO CO – CULTURAL SYSTEMS OF PRIMARY EQUINE ENDOMETRIAL EPITHELIAL AND STROMAL CELLS

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Introduction: For the approach to new and crucial insights into the pathogenesis of endometrial related fertility disturbances, an in-vitro culture system is highly suitable. Under standardised and reproducible conditions it ensures the best experimental opportunities from a practical and ethical point of view for controlled studies on the cellular level.

Material and Methods: On the basis of successfully established equine endometrial epithelial and stromal cell (EC/SC) monocultures, previously published methods were applied on 10 endometrial specimens of gynaecologically healthy mares to establish a co-culture system. As a modification the EC were cultured on one side of a semi-permeable membrane of a Millicell™-insert using serum-supplemented medium while the SC were cultivated separately for two days before being applied onto the other side of the same membrane. Confluent cultures were fixed in 4% neutral buffered formalin and processed routinely for histological examination. Culture purity of both cell layers was assessed using immunohistochemical expression of Cytokeratin 19.

Results: In accordance to data from monocultures the co-cultured cells formed confluent layers within 8 to 14 days. Moreover, consistent with findings in monocultures the EC showed explicit signs of epithelial differentiation in successfully established co-cultures with a continuous and considerable expression of Cytokeratin-19.

Conclusion: As EC and SC showed comparable morphological characteristics in mono- and co-culture, further analysis will include immunohistochemical examinations regarding the expression of hormone receptors, secretory proteins and structural filaments.

Notes:

Oral Presentations ESVP/ECVP

O03 PSEUDOPLACENTATIONAL ENDOMETRIAL HYPERPLASIA IN THE BITCH – A HISTOMORPHOLOGICAL AND IMMUNOHISTOCHEMICAL APPROACH

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Introduction: Pseudoplacentational endometrial hyperplasia (PEH) is a spontaneously occurring disease in bitches leading to infertility. Although the cause is unknown, it was shown to be inducible by various agents (oil, bacteria, suture material). PEH reveals a placenta-like morphology different from other types of endometrial hyperplasia. It is proposed that a prolonged progesterone influence after oestrogen-priming leads to PEH. The aim of this study was to characterise histochemical patterns and the expression of steroidhormone receptors and Ki67-antigen in PEH.

Materials and Methods: Twelve bitches (age 1 to 7 years) underwent ovariohysterectomy and samples were submitted for routine diagnostics. All samples were routinely processed for histology/histochemistry (HE, Alcian Blue, PAS) and immunohistochemistry (Ki67-antigen, Oestrogen- [ER], Progesterone-Receptor [PR]).

Results: All samples showed the specific morphology of PEH. The surface epithelium was homogenous and intensively positive for Alcian-blue and PAS, while the glandular epithelia reacted heterogeneously mild to moderate. An expression of PR in the surface epithelium and glands was absent or mild, whereas the stromal cells and the myometrium showed moderate staining intensity. The expression pattern of ER was similar but staining intensity was milder. Ki67-antigen positive cells were rarely seen in the epithelium (surface and glandular) and the stromal cells.

Discussion: This is the first study describing the hormone receptor expression in canine PEH. The expression pattern in PEH is similar to the findings in normal canine placenta described in the literature, indicating a local endocrine induced endometrial maldifferentiation.

Notes:

O04 COMPARISON OF METASTATIC PHENOTYPES IN MAMMARY TUMOURS OF THE DOG AND CAT

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Introduction: Recently the application of the molecular classification has allowed the identification of luminal (hormone receptor positive) and non-luminal (hormone receptor negative) phenotypes in both canine and feline carcinomas. Aim of the present work was to investigate the molecular phenotype in a set of canine and feline mammary carcinomas with positive regional lymph node.

Materials and Methods: Forty-eight canine and 57 feline mammary carcinomas with the corresponding lymph node metastasis were available for immunohistochemical analysis using a panel of antibodies (anti-ER, -PR, c-erbB-2, -P63, -CK14, -CK5/6, -CK19). The tumours and metastases were classified, according to a standardised algorithm, into one of the following 5 phenotypes, namely Luminal A and B; non-luminal: c-erbB-2 over-expressing, basal-like, normal-like.

Results: Canine primary tumour phenotypes evidenced a higher prevalence of luminal carcinomas (30/48) ($P<0.05$) while the cat showed a majority of c-erbB-2 over-expressing (22/57) ($P<0.05$). In lymph node metastases a significantly higher frequency of non-luminal phenotypes was evidenced in both species ($P<0.01$). In the dog, the basal-like (18/48) type was prevalent followed by the c-erbB-2 over-expressing (14/48) type, while in the cat the c-erbB-2 over-expressing (27/57) prevailed followed by the basal-like (17/57) type.

Discussion (and/or Conclusions): This investigation confirms that the main difference between mammary canine and feline carcinomas concerns the hormone receptor expression, which is higher in canine primary tumours. In both species when the initial site of metastasis is considered (regional lymph node) there is a shift from hormone positive to hormone negative phenotypes.

Notes:

Oral Presentations ESVP/ECVP

O05 TUMOUR-ASSOCIATED LEUKOCYTIC INFILTRATE IS RELATED TO HISTOLOGICAL CHARACTERISTICS AND PROGNOSIS IN CANINE MAMMARY TUMOURS

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Introduction: The role of tumour-associated leukocytic infiltrate (TLI) is mostly unknown in different types of neoplasms, including canine mammary tumours. Recent studies suggest a participation of tumour infiltrating lymphocytes (TILs), and tumour associated macrophages (TAMs) in tumour progression and prognosis with possible therapeutic implications.

Materials and Methods: Seventy-nine samples of surgically excised canine mammary tumours were prospectively collected. All samples were histologically diagnosed and graded. Immunohistochemistry of CD45, CD45RA, CD11b, CD11d, MHCII, CD3, CD4, CD8, CD21 (Prof. Peter Moore, UCDavis, USA) was performed on cryostat sections. Dogs were followed-up for 18 months.

Results: TLI was predominantly diffuse in benign tumours and focal (forming lymphoid pseudofollicles) in malignant tumours. TILs were significantly increased in malignant tumours although a severe reduction was observed in grade III tumours. TAMs increased with tumour malignancy. The most frequent leukocyte populations were lymphocytes (naïve CD45RA+ and T CD3+) and macrophages (CD11b+). Tumour epithelial and myoepithelial cells immunolabeled frequently for MHCII. B cells were detected in 65.1% of the malignant tumours, showing three types of pseudofollicles according to the cell types detected. In dogs with malignant tumours (n=43) the reduction of CD8+ T cells was associated to the development of metastases and recurrences, and the presence of B cells was associated to recurrences during follow-up (multivariant analyses).

Conclusions: According to our data TLI varies with histological characteristics, and the specific immune response is reduced in the most malignant tumours. The reduction of CTLs and the presence of B lymphocytes are independently related to a poorer prognosis.

Notes:

O06 LYMPHATIC EMBOLI OF FELINE INVASIVE MAMMARY CARCINOMAS: IMPROVED DETECTION USING IMMUNOHISTOCHEMISTRY

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Introduction: Lymphovascular invasion (LVI), the presence of lymphatic emboli on histological sections, is a strong prognostic factor in human, canine and feline mammary carcinomas. In uncertain cases, immunohistochemical markers of lymphatics such as D2-40 in human and prox-1 in canine oncology, may be used to assess LVI. The purpose of this study is to assess the usefulness of LMO2, a lymphatic endothelial marker, to improve LVI detection and prognostication in feline invasive mammary carcinomas (FMCs).

Materials and Methods: Retrospective study of 350 FMCs in female cats treated by surgery only, whose outcome is known 2 years post-mastectomy. Immunohistochemistry (IHC) to LMO2 was applied in 25 cases uncertain for LVI, and 38 cases that were free of visible emboli on HE-stained sections, including 16 cases without (No) and 22 cases with (N1) lymph node metastasis.

Results: Among LVI-uncertain cases, 17/25 (68%) were assigned to the LVI+ group according to LMO2 IHC. In the 38 cases free of HE-detected emboli, LMO2 IHC allowed to detect LVI in 4/16 No cases (25%) and 10/22 N1 cases (45%). In the whole cohort of 350 FMCs, LVI detected by routine HE histology was strongly associated with cancer-specific survival (HR=2.04 for the LVI+ group, $p<0.0001$) but there was no significant difference between LVI-uncertain and LVI-negative cases. Re-assignment using LMO2 IHC predicted a significantly lower risk of cancer-related death in LVI-negative cases (HR=0.31) than in LVI+ cases ($p=0.0001$; log-rank test).

Conclusions: The lymphatic endothelial marker LMO2 improves LVI detection and prognostication in cats with invasive mammary carcinoma.

Notes:

Oral Presentations ESVP/ECVP

O07 PATHOLOGIC NODAL STAGE OF FELINE INVASIVE MAMMARY CARCINOMAS

Notes:

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Introduction: In human breast cancer staging, the pathologic nodal stage (pN), determined by routine histology and pancytokeratin immunohistochemistry (IHC), is strongly associated with cancer-specific survival (CSS), i.e. the probability of dying from breast cancer. The aim of this study is to determine the prognostic value of the regional lymph node status, defined by the pathologist, in feline invasive mammary carcinomas (FMCs).

Materials and Methods: Retrospective study of 124 FMCs removed with the regional lymph node, in female cats treated by surgery only, whose outcome is known 2 years post-mastectomy. Pathologic nodal stage determined by haematoxylin-eosin and confirmed using cytokeratin (clone AE1/AE3) IHC.

Results: The pathologic nodal stage was pNo (no lymph node metastasis histologically, negative IHC) in 20/124 cats (16%), pNi+ (malignant cells ‹0.2 mm detected by HE or IHC) in 4/124 cats (3%), pNmic (micrometastasis, 0.2 to 2 mm in diameter) in 8/124 cats (7%) and pN1 (lymph node metastasis ≥0.2 mm) in 92/124 cats (74%). By multivariate analysis, CSS was significantly associated with the pathologic nodal stage (HR=0.45 for the pNo stage) as well as the size of the FMC (HR=0.40 for T‹20 mm), distant metastasis (HR=5.12 for the M1 stage), the histological type of FMC, the type of mastectomy and the spay status (HR=0.35 for cats spayed at the time of mastectomy).

Conclusions): In this series of 124 feline invasive mammary carcinomas, the pathologic nodal stages pNi+ and pNmic carried the same adverse prognosis as pN1 with respect to specific survival.

O08 PLASMA CIRCULATING CELL-FREE DNA AND APOPTOTIC MARKERS IN CANINE MAMMARY TUMOURS

Notes:

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Introduction: Early detection and reliable follow-up markers are crucial both in human breast cancer (HBC) and in canine mammary tumours (MT). Particular attention has been reserved to plasma circulating cell-free DNA (cfDNA) as a non-invasive test for early diagnosis/prognosis. The integrity of circulating DNA, measured as the ratio of longer to shorter DNA fragments, is higher in cancer-bearing patients and significantly related to prediction of metastasis.

Materials and Methods: In this study we analysed cfDNA from the plasma of 44 female dogs carrying malignant (36) and benign (8) MTs and from non-neoplastic controls (35). We also assessed markers of apoptosis (Bcl2, Bax and Bad) by immunohistochemistry and neoplastic morphological features.

Results: cfDNA was significantly higher in subjects carrying malignant tumours compared to those with benign lesions and controls. This seemed to be related with the frequent necrosis evidenced in malignancies. The analysis of apoptotic markers showed a diffuse increase of Bcl2 in tumours. Bcl2 was prevalent in the basal cells of non-neoplastic tissues whereas diffusely suprabasal/luminal in the neoplasia. High expression of Bax and Bad was detected only in multifocal neoplastic cells.

Discussion (and/or Conclusions): In this study we highlight that cfDNA might represent a significant marker of malignancy in canine MTs. Apoptosis and necrosis both cause DNA fragmentation with differently sized fragments. Here the cfDNA increase appears to be associated with the necrosis often evidenced within mammary carcinomas. In this study apoptosis seems to increase in cell clusters whereas the apoptosis inhibitor Bcl2 is diffusely overexpressed in tumours.

Oral Presentations ESVP/ECVP

O09 CD105 EXPRESSION AND USEFULNESS OF ITS TARGETING FOR ANTIVASCULAR ALPHA RADIOIMMUNOTHERAPY IN A MOUSE MODEL OF BREAST CANCER

Notes:

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Introduction: Radioimmunotherapy uses radiolabelled specific antibodies to selectively irradiate the tumour. Alpha particles are characterised by high energy and short path length and are particularly suitable for vascular targeting. CD105 overexpression is correlated with a poor prognosis in tumours and may be an interesting cellular target for preclinical research.

Materials and Methods: We evaluated a) the expression of CD105 in normal murine tissues and in xenografted tumour (MDAMB468); b) the biodistribution of an antibody raised against murine CD105 (MJ7/18) in xenografted mice; c) the effectiveness of a targeted radioimmunotherapy using the MJ7/18-213Bi in xenografted mice (MDAMB468).

Results: Immunohistochemistry studies showed that CD105 was strongly expressed on intratumoural capillaries. Biodistribution study showed an accumulation of MJ7/18 in the tumour, unlike other organs, in favor of antibody fixation and accumulation on tumour vasculature. Alpha radioimmunotherapy with 213Bi-MJ7/18 showed a delayed tumoural growth.

Conclusion: Targeting specifically tumoural vessels is crucial to avoid adverse toxic effects. So, it is of a great usefulness to test new more specific antigen, as CD105, to better target intratumoural vessels. These preliminary results demonstrated that targeting CD105 with alpha radionuclides seemed to be a promising anticancer strategy.

O10 PI3K SIGNALLING IN MOUSE MODELS OF INVASIVE LOBULAR BREAST CANCER

Notes:

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Introduction: Invasive Lobular Carcinoma (ILC) is a type of breast cancer composed of dyscohesive neoplastic cells lacking the intercellular adhesion protein E-cadherin. Activating mutations in PI3K signaling occur frequently in ILC. We have investigated the therapeutic potential of PI3K signaling inhibition, as well as the causality of PI3K signaling and E-cadherin loss in the pathogenesis of ILC.

Materials and Methods: We used a previously published genetically engineered mouse model of ILC, based on inactivation of E-cadherin and p53, to investigate preclinical in vivo benefit of PI3K signaling inhibition. We modeled neoadjuvant and adjuvant treatment in a realistic mouse cancer clinic setting, including surgery and monitoring of metastatic disease progression. At several stages of disease we performed proteomic and transcriptomic analysis on the tumour tissue.

Secondly, we engineered a new mouse model based on the activation of PI3K signaling by knocking out PTEN, a known tumour suppressor that counteracts PI3K signaling, and E-cadherin (alone and combined), followed by phenotypic analysis.

Results: Inhibiting PI3K signaling effectively suppressed progression of both primary tumours as well as metastases. In long term experiments, resistance developed. Using proteomics, transcriptomics and bioinformatics, we identified putative resistance mechanisms of ILC to PI3K signaling inhibition.

Genetic hyperactivation of PI3K signaling led to the formation of ILC-like metastatic tumours in mice, but only when combined with E-cadherin loss.

Discussion (and/or Conclusions): Results from our preclinical interventions support the role of PI3K signaling as both a therapeutic target as well as a key player in the pathogenesis of ILC.

Oral Presentations ESVP/ECVP

O11 HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL EVALUATION OF TWENTY SPONTANEOUS CANINE GLIOMAS SUGGESTS THE INVOLVEMENT OF CANCER STEM CELLS

Notes:

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Introduction: Diffuse gliomas, the most common primary brain tumours in humans, are associated with poor prognosis. An accurate animal model recapitulating tumourigenesis of human gliomas is needed to test new treatment strategies. In this context, dogs represent an interesting model as they develop spontaneous diffuse gliomas. In order to assess the relevance of the canine model, this study aims to evaluate if spontaneous canine gliomas contain cancer stem cells (CSCs) previously identified in all grades of human gliomas.

Materials and Methods: Twenty cases of canine gliomas were graded according to the human WHO classification. The expression of different markers of lineage differentiation was evaluated by immunohistochemistry (IHC): Nestin and CD133 for neural stem cells, doublecortin for neural progenitor cells, Olig2 for glial progenitor cells, Glial Fibrillary Acidic Protein, Vimentin and S-100 for glial cells, NeuN and βIII tubulin for neurons.

Results: Gliomas were characterised as follows: 5 grade-II (4 oligodendrogliomas, 1 oligoastrocytoma), 9 grade-III (8 anaplastic oligodendrogliomas, 1 anaplastic astrocytoma) and 6 grade-IV (glioblastoma). The IHC evaluation revealed that (i) the expression of S-100 protein and Olig2 did not differ substantially between astrocytomas and oligodendrogliomas, (ii) Nestin and CD133 were expressed in all grades of gliomas with a higher positivity in high-grade gliomas (HGG), (iii) all gliomas were negative for differentiated neuron markers while some HGG were detected positive for doublecortin.

Discussion (and/or Conclusions): Our results demonstrate the presence of CSCs in all grades of spontaneous canine gliomas, confirming the relevance of this animal model.

O12 CONTRIBUTION OF STEM CELLS TO BENIGN AND MALIGNANT CANINE PROSTATE TISSUES

Notes:

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Introduction: Canine prostatic carcinoma (PCa) is considered a relevant model for studying human advanced PCa. Survivin is proposed as a cancer biomarker for malignancy in human prostatic cancer. Sox9 is a stem cell marker expressed in several adult tissues, required for prostate development. Aims of the present study were to evaluate the patterns and levels of expression of survivin and Sox9 in canine benign prostatic hyperplasia (BPH) and PCa, and the potential co-localisation with androgen receptor (AR) and p63.

Materials and Methods: Immunohistochemistry and immunofluorescence with specific antibodies in a set of canine BPH and PCa.

Results: Survivin nuclear and rare cytoplasmic immunostaining were present among the reserve cell layer of normal and hyperplastic prostatic lobules. Increased survivin expression was observed in PCas compared with BPHs. Sox9 expression was absent in normal prostatic glands and in all BPHs. 6/9 cases of PCa were strongly positive. We observed a partial co-localisation of Surv-p63, Surv-AR in all the cases, and Surv-Sox9 in PCas.

Discussion (and/or Conclusions): Based on the stem cell role of survivin and the main role in proliferation of nuclear survivin, the survivin-positive cells in the reserve cell layer in normal and HBP cases could represent transit amplifying cells maintaining some stem cell proprieties. The increased survivin expression in PCas would indicate the molecule as a prognostic marker. The high Sox9 expression observed in PCas clearly suggests an important role of the molecule in canine prostatic carcinogenesis and malignant progression. Further studies should be done in order to confirm this hypothesis.

Oral Presentations ESVP/ECVP

O13 IMMUNOHISTOCHEMICAL PHENOTYPING OF CANINE PRIMARY CENTRAL NERVOUS SYSTEM NEOPLASMS USING TISSUE MICROARRAY

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Introduction: High-throughput tissue microarray (TMA) has been validated in a previous study as a useful technique for the simultaneous immunohistochemical phenotyping of large numbers of samples, especially in tumour research. In this study, TMA was used in order to evaluate the expression patterns of certain canine primary central nervous system (CNS) neoplasms and to identify potential markers with discriminative diagnostic relevance.

Materials and Methods: 97 primary CNS neoplasms, previously diagnosed on HE sections according to the WHO classification, were investigated on TMA with each tumour consisting of two cylindrical samples of 1.2 mm diameter from the center and periphery of the tumour. 28 monoclonal and polyclonal antibodies were used to phenotype the tumours and hierarchical clustering was applied to group the neoplasms according to their expression profile.

Results: Hierarchical clustering generally grouped cases with similar histological diagnoses, though the different gliomas exhibited a considerable overlap in their expression profile. Several general tumour groups such as gliomas, meningeomas, plexus tumours, ependymomas, germ cell tumours, and haematopoietic neoplasms significantly differed in the expression of certain markers such as $p75^{NTR}$, AQP_4, GFAP, and PLP.

Conclusion: TMA proved to be a useful material- and time-saving tool for the simultaneous immunohistochemical characterisation of canine CNS neoplasms. The present study provides a detailed overview of the expression patterns of different types of primary CNS neoplasms. Moreover, several novel antibodies with potential diagnostic relevance were identifiedd.

Notes:

O14 MITOTIC INDEX IS PREDICTIVE OF RECURRENCE IN FELINE INJECTION-SITE SARCOMAS (FISS)

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Introduction: Feline injection-site sarcoma (FISS) is an aggressive subcutaneous tumour developing a variable period after vaccination. It is believed to arise as a result of proliferation of fibroblasts and myofibroblasts at sites of chronic inflammation induced by injection. Histologically, FISS is characterised by inflammatory peritumoural infiltration, proliferation of atypical spindle cells and multinucleated giant cells. Gelatinases and TIMP-2 are enzymes pivotal in extracellular matrix remodeling and therefore in tumour invasiveness. SMA expression is reported in myofibroblastic cellular phenotype of human soft-tissue sarcomas, where it is associated with a favorable prognosis. Aim of this study was to investigate different macroscopic, histologic and immunohistochemical features of FISS to evaluate their usefulness as prognostic indicators of recurrence.

Materials and Methods: 24 cats with histologically confirmed FISS were classified according to clinical evolution during 1-year follow-up in non-recurrent (NR, n= 15) and recurrent (R, n= 9) FISS. Different macroscopic and histologic parameters were evaluated; soft-tissue sarcomas grading was applied. Immunohistochemical expression of vimentin, MMP-2, MMP-9, TIMP-2 and SMA was evaluated.

Results: Among histologic parameters, only the number of mitoses/HPF was associated with FISS recurrence ($P<0.001$). The cut-off value of 2 mitoses/ HPF showed a sensitivity of 78%, a specificity of 87% and an accuracy of 83% in predicting the recurrence risk. Immunohistochemistry did not show noteworthy differences between R and NR groups.

Discussion (and/or Conclusions): Our study confirms that the current grading system is not predictive of the risk of FISS recurrence, whereas the number of mitoses/HPF can be considered as a useful prognostic parameter.

Notes:

Oral Presentations ESVP/ECVP

O15 CREDENTIALING NATURALLY OCCURRING CANINE MELANOMA AS A SURROGATE MODEL FOR HUMAN DISEASE

Notes:

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Introduction: Melanoma represents a significant malignancy in humans and dogs. Study of sporadic canine melanocytic neoplasms has several advantages over genetically engineered models, including tumour diversity, functional immunity and clinical management. A tumour board including medical and veterinary pathologists evaluated clinical and pathologic correlations between human and canine mucosal melanomas and concluded clinical trials in dogs could be a promising surrogate model. Additional assessment for coinciding pathway activation may provide insight into common molecular derangements suitable for targeted therapies.

Materials/Methods: Phosphorylation of nodes in AKT and MAPK signaling pathways was investigated by immunohistochemistry on tissue microarrays containing 44 canine and 37 human primary mucosal melanomas. Colour deconvolution digital image analysis was used to compare expression levels between tumour samples and canine oral melanocytic neoplasms with low malignant potential (LMP). Canine melanomas were sequenced for orthologous BRAF and NRAS mutations.

Results: Most canine and human mucosal melanomas showed activation of the AKT (95% and 89 %), MAPK (73% and 43%) and mTOR (100% and 95%) pathways, respectively. 52% dog and 43% human melanomas exhibited co-activation of multiple pathways. Pathway activation was minimal in canine LMP tumours. Two primary canine melanomas harbored NRAS mutations.

Discussion: Activation of AKT and MAPK signaling is common in both canine and human mucosal melanoma, although BRAF and NRAS mutations are uncommon in canine melanoma. Comparisons of pathway activation levels between patients are complicated by sample/tumour heterogeneities, analysis methods, and to some degree pigmentation despite digitally subtracting melanin from tissue image expression intensity measurements. Results support canine oral melanoma as a rational spontaneous model of melanoma.

O16 PASSAGE-DEPENDENT MORPHOLOGICAL AND PHENOTYPICAL CHANGES OF A CANINE HISTIOCYTIC SARCOMA CELL LINE (DH82-CELLS)

Notes:

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Introduction: DH82-cells are a permanent canine macrophage cell line isolated from a dog with histiocytic sarcoma. Numerous studies render DH82-cells as an appropriate *in vitro* model for the investigation on macrophage behavior in cancer research and infection medicine. Since, existing data on cell surface antigen expression are fragmentary and in part inconsistent we aimed at a detailed antigenic and morphological characterisation of canine DH82-cells with respect to passage-dependent differences.

Materials and Methods: Early (‹ 13) and late (› 66) passages of DH82-cells were maintained under standard culture conditions and labelled with 10 monoclonal antibodies directed against CD11c, CD14, CD18, CD44, CD45, CD80, CD86, MHC-I, MHC-II, and ICAM-1 for flow cytometric analysis of cell surface marker expression. Cellular morphology was descriptively evaluated via scanning electron microscopy.

Results: The expression of CD11c, CD14, CD18, CD45 and CD80 significantly decreased in late passages whereas the expression levels of CD44, CD86, MHC-I, MHC-II and ICAM-1 remained constant. DH82-cells of early and late passage displayed morphological differences with early passages presenting as roundish cell bodies with abundant small cytoplasmic projections whereas later passages exhibited a spindleoid morphology with scant but large processes.

Conclusion: DH82-cells represent a remarkably heterogeneous macrophage cell line with distinct antigenic and morphological properties. The passage-dependent decline in the expression of various surface antigens might explain partly diverging reports on the expression profile of DH82-cells. Thus, the present findings have important implications for future studies, which should consider distinct characteristics, depending on the used passage.

Oral Presentations ESVP/ECVP

O17 TECTONIN BETA-PROPELLER REPEAT-CONTAINING PROTEIN 2 (*TECPR2*) MISSENSE MUTATION ASSOCIATED WITH NEUROAXONAL DYSTROPHY IN PERRO DE AGUA ESPANOL

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Introduction: Neuroaxonal dystrophies (NAD) in humans and animals define a heterogeneous group of inherited or acquired neurodegenerative disorders. In humans but not in domestic animals, a variety of underlying mutations have been characterised.

Materials and Methods: Four related Perros de Agua Español aged from 1 to 1.5 years with neurological disorders were submitted for necropsy. Further examination encompassed histology, immunohistology and transmission electron microscopy. Genetic analysis included single nucleotide polymorphism (SNP) array genotyping and whole genome re-sequencing. Based on the assumption of autosomal recessive inheritance the four cases were analysed for extended regions of shared homozygosity and linkage analysis was performed. Whole genome re-sequencing data of one affected and 29 control dogs were used for mutation analysis.

Results: Clinical features included gait abnormalities, disturbances of peripheral reflexes and visual deficits. Histology, immunohistology and electron microscopy revealed spheroid formation within the CNS as well as intraspheroidal accumulations of neurofilaments, mitochondria and ubiquitinated protein aggregations. Genetic analysis identified a highly damaging tectonin beta-propeller repeat-containing protein 2 (*TECPR2*) gene missense mutation (c.4009C>T; p.R1337W) as the cause for NAD in Perros de Agua Español.

Discussion: NAD affected dogs displayed aetiological, clinical, and histological similarities to a variant of recessive inherited human hereditary spastic paraparesis caused by a TECPR2 mutation. TECPR2 is a poorly characterised protein of the autophagic pathway. Therefore NAD in Perros de Agua Español may provide an animal model to study TECPR2 function, pathogenesis of NAD and the role of the autophagy pathway in the pathogenesis of neurodegenerative diseases.

Notes:

O18 COMPLEX AXONAL TRANSPORT DISTURBANCES AND EVIDENCE OF SCHWANN CELL REMYELINATION IN CDV-INDUCED DEMYELINATING LEUKOENCEPHALITIS

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Introduction: *Canine distemper virus* (CDV)-induced demyelinating leukoencephalitis is characterised by a progressive axonal injury resulting in extensive axonal loss. However, the mechanisms contributing to axonal injury and the interactions between demyelination and axonal loss as well as possible remyelinating processes still remain undetermined.

Materials and Methods: Cerebellar tissue from dogs naturally infected with CDV was classified according to the age of the lesions and compared to neurologically healthy controls. Samples were stained with haematoxylin and eosin and further processed for immunohistochemistry using antibodies directed against axonal skeletal and transport proteins as well as marker proteins for Schwann cells. Additionally, the regulation of manually selected genes, implied in axonal pathology, was analysed in a dataset of a microarray analysis of dogs with CDV encephalitis.

Results: In CDV encephalitis, an early and progressively decreased expression of cytoskeletal constituents (neurofilaments and tubulins) as well as of motor proteins (kinesin, dynein and tau-1), which could in part be recapitulated on the transcriptome level, was detected. Neurotrophin receptor $p75^{NTR}$ expressing cells were observed within subacute and chronic CDV lesions and were absent in the non-affected white matter. Periaxin immunoreactivity was detected in two CDV cases.

Discussion (and/or Conclusions): Summarised, there is evidence of early arising disturbances of the axonal cytoskeleton resulting in a defective axonal transport and finally leading to a widespread and potentially irreversible loss of axons in CDV encephalitis. Besides a presumably insufficient, not further investigated oligodendroglial remyelination in CDV so far, there is now indication of Schwann cell recruitment and remyelination.

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Oral Presentations ESVP/ECVP

O19 AXONAL PATHOLOGY AND AXONAL REGENERATION MECHANISMS IN CANINE SPINAL CORD INJURY

Notes:

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Introduction: Canine spinal cord injury (SCI) caused by intervertebral disk disease (IVDD) results in a devastating neurological disease. Axonal pathology in canine SCI has been described but remains incompletely understood in terms of detailed pathomechanisms. This study aimed at a characterisation of axonal cytoskeletal and transport alterations as well as possible endogenous factors contributing to axonal regeneration.

Materials and Methods: Spinal cords of dogs with spontaneous acute and subacute IVDD were compared to controls. Axonal cytoskeletal components and molecules contributing to promotion of axonal growth were evaluated quantitatively by means of immunohistochemistry applying various monoclonal and polyclonal antibodies. These investigations were substantiated by analysis of a manually generated list of potential marker genes implied in axonal pathology and regeneration in a microarray data set of the same dogs.

Results: As an expression of significant axonal transport and cytoskeletal disturbances, marked axonal accumulation of axonally transported proteins (e.g. dynein, kinesin) and cytoskeletal constituents (e.g. tubulins) was detected. In parallel, factors contributing to axonal regeneration (e.g. EPO, HIF-1α) were demonstrated to be up-regulated in both acute and subacute SCI. Several analogous genes proved to be differentially regulated on the transcriptome level.

Conclusions: The results underline significant axonopathy as an important feature of the early phase of IVDD. As the accumulation of cytoskeletal and transport components in injured axons may represent either inhibitory or supporting factors for axonal regeneration, the functional consequence remains to be determined in the future. The demonstrated up-regulation of endogenous regeneration promoting factors might represent a promising therapeutic target.

O20 P75^{NTR}-EXPRESSING CELLS WITH REMYELINATING POTENTIAL IN SPONTANEOUSLY OCCURRING CENTRAL NERVOUS SYSTEM DISEASES IN DOGS

Notes:

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Introduction: After central nervous system (CNS) injury, oligodendrocyte precursor cells may differentiate into mature oligodendrocytes and CNS Schwann cells (SCs). The role of CNS-derived SCs in spontaneous CNS diseases is not known. In this study, the occurrence of p75 neurotrophin receptor (p75^{NTR}) and periaxin-immunoreactive cells as markers for pre-myelinating and myelinating SCs, respectively, was investigated in different spontaneous canine CNS conditions and linked to the degree of inflammation and axonal damage.

Materials and Methods: Dogs suffering from spontaneous degenerative CNS diseases (n=10), suppurative meningoencephalitis (n=8), granulomatous meningoencephalitis (GME, n=19), neoplasia (n=5), and lymphohistiocytic meningoencephalitis (n=7) were processed for histology, as well as for lectin- and immunohistochemistry using the lectin of *Bandeiraea simplicifolia* 1 and antibodies directed against p75^{NTR}, periaxin, amyloid precursor protein, CD3, and glial fibrillary acidic protein.

Results: The diseases showed a variable degree of axonal pathology, gliosis and inflammation, respectively. p75^{NTR} immunoreactive bi- to multipolar cells, suggestive of pre-myelinating SCs, were found intralesionally in 13 out of 19 of GME cases (68%), but only rarely in the other conditions. Remyelinating periaxin positive SCs were almost exclusively observed in GME (5 cases, 26%).

Discussion: The results demonstrate that several canine CNS diseases are accompanied by emergence of p75^{NTR}-positive, potentially growth-promoting and pro-regenerative glia. GME was identified as a unique disease entity with enhanced occurrence of such cells, suggesting a link between p75^{NTR} expression and a particular inflammatory microenviroment. Furthermore, intralesional expression of periaxin demonstrates effective endogenous SCs remyelination in GME.

Oral Presentations ESVP/ECVP

O21 COCULTURES OF CANDIDATE CELLS FOR TRANSPLANTATION STUDIES AFTER SPINAL CORD INJURY WITH NEURONS – HOW TO SORT THE WHEAT FROM THE CHAFF

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Introduction: Spinal cord injury (SCI) is one of the most frequently occurring diseases of the central nervous system in dogs. So far, efficient therapeutics are lacking for severe cases of SCI in dogs and also in humans. Cell transplantation approaches represent a promising method to cure severe cases of SCI. Dorsal root ganglion neurons (DRG) represent an urgently needed first step investigation method to detect axonal regeneration supporting properties of potential candidate cells for cell transplantation studies after SCI.

Materials and Methods: DRGs were obtained from healthy dogs and neurons were isolated. They were cocultured with several canine glial cells including peripheral and central Schwann cells and olfactory ensheating cells derived from the olfactory bulb or the olfactory mucosa, respectively, and non-glial cells *in vitro* to measure the amount of neurite outgrowth and arborisation.

Results: Glial cells exhibited a positive effect on neurite formation and branching of DRG neurons following coculture *in vitro* in comparison to mesenchymal cells.

Discussion (and/or Conclusions): Isolated DRG neurons cultured together with promising candidate cells for transplantation after SCI revealed an axonal growth promoting effect of the glial cells. This coculture system provides a promising tool to investigate axonal growth promoting effects of potential candidate cells for cell transplantation studies.

Notes:

O22 INTERLEUKIN-10 RECEPTOR BLOCKADE IN THEILER´S MURINE ENCEPHALOMYELITIS

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Introduction: Theiler´s murine encephalomyelitis (TME) is an important model for demyelinating diseases in animals and humans. The aim of the present study was to determine the influence of the immunosuppressive cytokine interleukin-10 on the course of TME in SJL mice.

Materials and Methods: Five week old SJL mice were intracerebrally infected with TME virus and received anti-IL-10 receptor antibody (anti-IL-10R Ab) intraperitoneally on day 0, 7, 14 and 21 after infection. Control animals received an IgG isotype specific antibody (n=5 in each group). Brain, spinal cord and peripheral organs were examined histologically and immunohistologically. Viral load has been determined by immunohistochemistry and peripheral blood has been examined by flow cytometry.

Results: TME virus infected animals with and without anti-IL 10R Ab treatment developed meningomyelitis with evidence of virus infected cells in the white matter. Anti-IL-10R Ab treatment causes severe lymphohistiocytic colitis in treated animals and phenotypic variations in the peripheral blood. On day 22, $CD19^+$ B cells were decreased in the peripheral blood whereas an increase of $CD4^+CD44^+$ and $CD8^+CD69^+$ T cells has been determined. Despite this upregulation of effector/memory T cells and downregulation of B cells in the periphery, no differences in the central nervous system (CNS) between the groups have been determined by histology.

Discussion: Results of the present study suggest that peripheral modulation of the IL-10 pathway plays a minor role for CNS immune response and neuroinflammation in TME, in contrast to the importance for the maintenance of mucosal tolerance.

Notes:

Oral Presentations ESVP/ECVP

023 EXPANSION OF REGULATORY T CELLS IN *THEILER'S MURINE ENCEPHALOMYELITIS VIRUS*-INFECTED C57BL/6 MICE

Notes:

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Introduction: *Theiler's murine encephalomyelitis virus* (TMEV) infection is a widely used mouse model for demyelinating disorders. Susceptible SJL mice develop a progressive demyelinating disease in the central nervous system (CNS) with virus persistence while resistant C57BL/6 mice rapidly clear the virus due to strong antiviral immune responses.

Materials and Methods: In order to investigate the effect of regulatory T cells (Treg) upon virus-specific immune responses, Treg were expanded in TMEV-infected C57BL/6 mice by the intraperitoneal application of interleukin-2 immune complexes (IL-2C). Antiviral cytotoxicity was reduced by CD8-depleting antibodies. At necropsy, CNS tissue was removed for histology, immunohistochemistry and molecular analyses. Phenotypical changes of the spleen were determined by flow cytometry.

Results: IL-2C-treatment leads to a peripheral induction and enhanced CNS-infiltration of $Foxp3^+$ Treg. Additional CD8-deplAETIOn causes prolonged TMEV infection of the spinal cord and leukomyelitis in C57BL/6 mice.

Discussion: Results of the present study demonstrate the occurrence of synergistic effects between Treg-expansion and depletion of cytotoxic T cells for the reduction of TMEV-specific immunity, leading to spinal cord inflammation in resistant mice. The induction of virus persistence will enable the investigation of virus-induced immunopathology in transgenic mice with a C57BL/6-background in future studies.

024 SPONTANEOUS AND EXPERIMENTAL RYEGRASS POISONING IN SANTA CATARINA STATE, BRAZIL

Notes:

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Introduction: Ryegrass (*Lolium* spp.) poisoning in dairy cattle has been occurring in southern Brazil in the last few years, particularly at the end of winter when the pasture presents exuberant growth after swine slurry application on the soil. History of sudden death is common and the need for definitive diagnosis is essential.

Materials and Methods: In 2013, five spontaneous cases of sudden death in dairy cattle on Ryegrass pasture were presented to this lab. Epidemiologic data, clinical signs and lesions were recorded. One cattle was experimentally poisoned to demonstrate the disease.

Results: Adult cattle were presented dead or alive. Within an average of four hours after consuming the pasture, the animals showed brownish mucosae, tachycardia, dyspnoea, ruminal bloating and recumbence. Experimental poisoning was reproduced after consumption of 4.5% of the body weight (BW) in 2 hours. Blood level of methaemoglobin was 100% after 3 hours. Later bromatological analysis showed 5% nitrate in dry matter. Two animals of the spontaneous and the experimental cases recovered completely after three hours under methylene blue 1% intravenous therapy (10mg per kg of BW).

Discussion (and/or Conclusions): For pathologists, reaching a conclusion on a sudden death can sometimes be a challenge. In this case, a difenilamine assay was used to test the presence of nitrate in the pastures, and together with the brownish colour of mucosae and blood, a conclusion was established. Our laboratory also diagnosed a cianidric acid poisoning in the same year. Picric acid assay was useful to differentiate the two conditions.

Oral Presentations ESVP/ECVP

025 FUMONISINS TOXICOSIS IN HORSES

Notes:

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Introduction: Natural outbreaks of clinical toxicosis from fumonisins have only been reported in horses and swine, with horses being the more susceptible species. The main target organ is the brain of horses and disease is known as equine leukoencephalomalacia (ELEM). Equine leukoencepalomalacia has been reported worldwide, but there are only few reports of the outbreak of ELEM in the Europe.

Materials and Methods: The head of the dead colt, Lipizzan breed, 18 months old, was examined. The problem arose in a stable which houses 100 horses, the animals started to be ill showing neurological symptoms and after 2 to 5 days died (15 out of the 21 affected). The brain was fixed *in toto* and cross- sectioned at various level. Tissue samples from the brain were processed for histologic examination. For determination of mycotoxins in feedstuffs, liquid chromatography was used.

Results: Macroscopically, cross sections of the brain revealed large areas with necrosis and softening of the brain white matter. Histological investigation showed necrosis and layering of the brain mass due to accumulation of fluid, infiltration of macrophages, neutrophils and eosinophils, haemorrhage and oedema of the surrounding gray matter. Fumonisins were disclosed in corn bran in a concentration of 6.05 mg / kg (FB1), and 1.87 mg / kg (FB2).

Conclusions: The established changes in the brain of the dead horse, the history data, as well as the clinical picture of the animal before death and isolation of mycotoxins fumonisin FB1 and FB2, point unequivocally to equine leukoencephalomalacia.

026 PATHOGENIC INFLUENCE OF DELTAMETHRIN ON INNATE IMMUNITY IN FISH

Notes:

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Introduction: Deltamethrin (98% cis isomer) is one of the most popular pyrethroid ester insecticides and it is used for control of ectoparasites in animals (sheep, poultry, cattle, piglets). Also it is used for rainbow trout and Atlantic salmon to treat sea lice (*Lepeophterus salmonis, Caligus elongatus*).

Materials and Methods: In our study we examined the influence of recommended bath dosages of deltamethrin on the innate immunity in rainbow trout. The healthy rainbow trout (*Oncorhynchus mykiss*), of weight 50-70 g were examined. The fish were held in 500 L tanks in 14 °C spring water and fed twice daily with commercial pellets. The fish were bathed in deltamethrin at concentration: 1, 2, 3 and 4 µg/L for 30 minutes in a closed glass container. The blood, pronephros and spleen from each bath groups and control group were isolated before and 1, 2, 5 and 7 days after bath for immunological study. The following techniques were used in the study of the non-specific cellular and humoral defence mechanisms in these experiments.

Results: On days 1, 2 and 5 after immersion with deltamethrin at each examined doses, the spleen macrophage and pronephros lymphocytes activities were significantly decreased ($P<0.05$), compared to the control group. Also the lysozyme activity and Ig levels in serum were significantly ($P<0.05$) decreased at days 5 and 7, compared to the control. The highest immunosuppression effect at dose 4 µg/L was observed.

Conclusions: The results of this study showed that deltamethrin at recommended doses induced immunosuppression on the cellular and humoral innate immunity in rainbow trout.

Oral Presentations ESVP/ECVP

027 COMPARISON OF ACID BIOCIDES INFLUENCE: STERDIAL W-15 AND DISINFECTANT CIP ON SKIN OF RAINBOW TROUT *(ONCORHYNCHUS MYKISS)*

Notes:

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Introduction: Acid biocides are recognised as highly efficient, safe for fish and totally biodegradable. These substances are based on organic acids and hydrogen peroxide.

Materials and Methods: In examination performed on fry of rainbow trout, mass 100 g, test items were used in concentrations: 8 mg/l of water. Concentrations of Steridial W-15 (s8) and CIP (c8) chosen for experiment, contained in doses range of preventive treatment. Samples of the skin were collected, fixed in 4% neutral buffered formaldehyde, embedded in paraffin, and stained with haematoxylin-eosin (HE) and alcian blue-periodic acid Schiff's reagent-haematoxylin (PAB).

Results: The major skin change in exposed group revealed by microscopic examination was a clearly reduction of the epidermis, hyperplasia of the epidermal cells, hypoplasia of mucous cells and desquamation of epithelium. In addition, a vacuolation of epithelial cells (c8) and leucocyte infiltration in the epidermis (c8) were found. Examined samples have shown changes at the level of the epidermis, without major changes in the dermis and hypodermis. It appears that both agents have equal efficacy and in this concentrations are showing big irritant properties.

Conclusions: In evaluation of biocides, we take into consideration efficacy of action against the pathogenic factor, toxicity for fish and other aquatic organisms. Biocides vary between each other with range of action and effect of physiological processes. In the light of presented results, important is the effect on tissue of skin and gills. In practical terms it is important to use those products for shorter time intervals and with great caution, to eliminate side effects.

028 MORPHOLOGICAL EVALUATION OF THE LIVER IN RAINBOW TROUT *(ONCORHYNCHUS MYKISS* WALBAUM, 1972) AFTER KYNURENIC ACID (KYNA) EXPOSITION

Notes:

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Introduction: KYNA is produced in the body by metabolic pathway for tryptophan. This amino acid is not synthesised in the body, but it may be provided by food containing tryptophan. The aim of this research was to evaluate the impact of KYNA on liver in rainbow trout by histopathological examination.

Materials and Methods: The experiment was conducted on 24 (n=6) rainbow trout with the average body mass 150.0 g. Animals were randomly divided into groups: 1 – control fish fed with commercial feed without a supplement of KYNA and 3 treatment groups with the addition of KYNA (SIGMA-ALDRICH) in doses (2) 2.5 mg/kg, (3) 25.0 mg/kg and (4) 250.0 mg/kg equal of 1% of the body mass. Liver were stained with HE.

Results: In the liver of the control group steatosis simplex of hepatocytes was observed. These lesions were significantly less evident in treatment groups: 2 and 3. In group 4 steatosis simplex was rarely found. Additionally, in liver of treatment group 2 abnormalities of Kupffer cells and single melanomacrophages were observed. In groups 3 and 4 lymphoid cells infiltrations were also found. In group 4 of KYNA foci of eosinophilic necrosis, Kupffer cells hyperplasia and hyperaemia were reported.

Discussion and Conclusion: The research results showed that higher doses of KYNA in feed caused a decrease of liver steatosis in rainbow trout. Kynurenic acid in dose of 250.0 mg/kg may cause eosinophilic necrosis and hyperaemia in liver of rainbow trout.

Oral Presentations ESVP/ECVP

029 EFFECT OF GLUCAN AND LOW DOSES OF T-2 TOXIN ON MUCIN, IgA EXPRESSION AND IgA+ LYMPHOCYTES IN THE INTESTINE OF CHICKENS

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Introduction: T-2 toxin is a mycotoxin and in low doses suggests to be stimulator of the immune system. The major class of Ig produced at mucosal surfaces is IgA. This immunoglobulin is known to interact with mucins through the secretory component. To detect effect of T-2 toxin during administration of β-D glucan on mucin and production of IgA we examined intestinal mucin (MUC2) and IgA gene expression and quantity of IgA+ cells.

Materials and Methods: Day old chickens (n=20) were divided into four groups: T-2 toxin (145 μg pure/1kg diet; from 14 to 28 day of age), β-D-glucan (3mg/chicken; consequently 11, 12, 21 day of age), β-D glucan+T-2 toxin and controls. Followed primers were used for RT-PCR: MUC2 forward-5´GCT GAT TGT CAC TCA CGC CTT; MUC2 reverse 5´ATC TGC CTG AAT CAC AGG TGC; IgA forward 5´GTC ACC GTC ACC TGG ACT ACA; IgA reverse 5´ACC GAT GGT CTC CTT CAC ATC. Flow cytometry: mouse anti-chicken IgA-PE labelled MoAb was used.

Results: T-2 toxin significantly increased mRNA of MUC2 in jejunum comparing to glucan+T-2 toxin group and controls. T-2 toxin also significantly upregulated mRNA of IgA in T-2 and glucan+T-2 toxin groups. Number IgA+ cells were unchanged in jejunal lamina propria in followed groups, however IELs significantly decreased in T-2 group.

Conclusions: T-2 toxin low dose upregulated mRNA of MUC2 and IgA in jejunum. Administration of glucan influenced only mRNA of MUC2 gene and number of IELs. Non stimulated IgA+ cells with T-2 toxin suggest only the higher production of IgA.

Notes:

030 COMPARISON BETWEEN ABV2 AND ABV4 DISTRIBUTION PATTERNS IN EXPERIMENTALLY INFECTED COCKATIELS

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Introduction: Proventricular dilatation disease (PDD) is a fatal disease characterised by a non-purulent enteric ganglioneuritis and encephalomyelitis. To date pathogenic differences of the 12 different ABV genotypes remain unknown. Thus,we characterised the inflammatory lesions and different distribution patterns of genotype ABV2 and ABV 4 in an experimental trial.

Materials and Methods: 18 cockatiels were infected intracerebrally and intravenously with ABV2 and 18 cockatiels with ABV4. Tissue samples were collected from the central nervous system (CNS), gastrointestinal tract (GIT) and all peripheral organs for histology, viral antigen and RNA detection by immunohistochemistry or in situ hybridisation, respectively.

Results: After ABV2 infection, inflammatory lesions and viral antigen were mainly found in the GIT and in the peripheral organs starting from day 46pi until the end of the investigation at day 231pi. Genomic ABV4 RNA and ABV4 mRNA were detected in the GIT while only viral mRNA were identified in the heart, liver, kidney, skin and skeletal muscles during the whole investigation period. After ABV4 infection, inflammatory lesions, viral antigen, genomic ABV4 RNA and ABV4 mRNA were found in the CNS and in the peripheral organs starting from day 60pi until the end of the investigation at 230pi. Abundance of viral mRNA was higher than presence of viral genome in the CNS while more genomic RNA than mRNA of ABV4 was found in the eye, heart, liver, kidney and skeletal muscles.

Conclusions: ABV2 and ABV4 infections can both cause PDD. However, ABV2 demonstrated a higher affinity towards the GIT and peripheral organs while ABV4 displayed a higher tropism for the CNS and peripheral organs.

Notes:

Oral Presentations ESVP/ECVP

031 SHEDDING OF INFECTIOUS *BORNA DISEASE VIRUS* IN LIVING BICOLOURED WHITE-TOOTHED SHREWS

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Introduction: Bicoloured white-toothed shrews have been recently confirmed as reservoir for Borna Disease, a fatal neurological disorder of horses and sheep. Natural infections with *Borna Disease Virus* (BDV) in three living bicoloured white-toothed shrews were investigated and shedding of virus was characterised.

Materials and Methods: Samples of saliva, lacrimal fluid, skin surface, urine and excrements from three natural BDV-infected shrews were taken. BDV RNA was amplified and quantified and infectious virus was isolated on rabbit embryonic brain cells. Results were compared to antigen and viral RNA distribution in tissue visualised by immunohistochemistry and in situ hybridisation.

Results: BDV-RNA was detected by RT-PCR in excretions and secretions such as saliva, urine, sebum, lacrimal fluid and faeces. Infectious virus was isolated from saliva, sebum and urine. These detections correspond well to the morphological demonstration of viral antigen and RNA in the respective organ systems.

Discussion (and/or Conclusions): Shedding of BDV in the bicoloured white-toothed shrew is achieved via various routes which enables successful viral maintenance in the reservoir population and even fatal transmission to susceptible end hosts such as horses.

Notes:

032 OUTBREAK OF RANAVIRAL DISEASE IN IMPORTED NEWT FROM CHINA

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Introduction: Epidemics of ranaviral disease have been reported in both free-ranging and captive amphibians in many parts of the world. The international pet trade is believed to have increased the spread of the pathogen. The purpose of this study is to report the epidemic of ranaviral diseases in newts imported from China.

Materials and Methods: Three *Tylototriton kweichowensis* that were imported from China and two previously inhabiting *T. kweichowensis* were housed together. All five animals died within 4 days after cohabitation. Further, two *T. kweichowensis* and four *T. taliangensis* died within 3 days after purchase. These dead newts were examined pathologically, and presence of ranavirus was tested using kidney tissue and PCR methods.

Results: Affected animals showed lethargy, gloom, and systemic oedema with severe swelling in the jaw. Gross findings showed body cavity oedema, hepatic enlargement, and spleen atrophy. Necrotic change in the parenchyma of organs was dominant histologically. In some cases, intra-cytoplasmic basophilic inclusion bodies were present in hepatocytes, epithelial cells of the kidneys and skin, haematoietic cells, and cardiomyocytes. FV-3 was detected in all animals. In addition, we found that these animals were from the same pet shop.

Conclusions: Tylototritons are a primitive type of newts and are protected as an endangered species. This ranaviral disease process is para-acute and lethal, and therefore this pathogen could become be a threat for this genus. This report emphasises the role of pet trade in the diffusion of these pathogens. To our knowledge, this is the first report of a ranavirus infection in imported animals of the Salamandridae family in Japan.

Notes:

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033 CLINICAL, MACROSCOPIC AND HISTOLOGICAL FEATURES OF CONTAGIOUS OVINE DIGITAL DERMATITIS (CODD)

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Introduction: Contagious Ovine Digital Dermatitis (CODD) is a recently identified, important cause of lameness in sheep in the UK. Currently CODD is poorly defined in terms of clinical presentations, and there has been no detailed description of the macroscopic or histopathological lesions. This work describes the pathological changes of different stages of this disease.

Materials and Methods: The affected feet from 1-2 sheep with clinical signs of CODD in each of 6 clinical stages were sampled and histological sections made from digital skin, coronary band, dorsal hoof wall and laminae, solar wall, and third phalanx. Sections were stained with Haematoxylin-Eosin and Warthin-Starry

Results: The early stage of the disease is represented by dermatitis of the digital skin and coronary band and is predominantly lymphoplasmacytic. In the most severe stage the cornified layer the dorsal hoof wall exhibits severe suppurative inflammation, haemorrhage and intra-lesional bacteria, and there is separation of the hoof wall from the underlying laminae. In the later, healing stages of the disease, the hoof wall re-grows but is often deformed. Milder histological lesions of lymphoplasmacytic dermatitis of the coronary band and suppurative inflammation of the horn remain. The dorsal aspect of the third phalanx exhibits a moderate degree of periosteal activation and osteophyte formation. Warthin-Starry stain reveals the presence of spirochaetal organisms morphologically consistent with *Treponema* sp.

Discussion (and/or Conclusions): This is the first description of the pathological changes of CODD, and demonstrates the presence of what is considered the most likely aetiological agent i.e. *Treponema* sp.

Notes:

034 MOLECULAR RETROSPECTIVE STUDY OF CANINE INFECTIOUS HAEMOLYTIC ANAEMIAS

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Introduction: Most common infectious agents of haemolytic anaemia in Croatian dogs are *Babesia canis* and *Leptospira interrogans*. Despite varied causes, post-mortem findings include anaemia, icterus, splenomegaly, and haemoglobinuric nephrosis. The aim of the study is to genotype pathogens in archival samples from dogs that died of haemolytic anaemia.

Materials and Methods: Slices, 30 µm thick, from formalin fixed paraffin embedded spleen, lungs, myocardium and kidney samples from 19 dogs were selected for molecular analyses. After incubated with xylene followed by ethanol washes, DNA was extracted with a commercial kit. All samples positive with mammalian cytochrome-C were screened for presence of *Leptospira* spp., *Babesia* and *Theileria* spp., *Anaplasma* and *Ehrlichia* spp., *Hepatozoon canis* and *Bartonella* spp. Amplified samples were purified and sequenced. Obtained results were compared with patho-anatomical, histopathological, cytological and serological findings.

Results: *Babesia* sp. found by post-mortem cytology in seven cases was confirmed in four dogs by PCR from lungs and myocardium, but not spleen. Sequencing revealed *B. canis* in three dogs and *Theileria* sp. in a single dog. Co-infection of *B. canis* and *A. phagocytophilum* was found in one myocardium. *Candidatus* Neoehrlichia mikurensis was detected in kidneys from a dog, and *A. phagocytophilium* in kidneys and lungs from another.

Discussion (and/or Conclusions): *Babesia canis* was the most frequent pathogen as expected. The presence of *A. phagocytophilum, Candidatus* N. mikurensis and *Theileria* sp. presents an unexpected finding in organs from dogs died from haemolytic anaemia. These findings suggest a complex aetiology of haemolytic anaemia in dogs without pathognomonic lesions characteristic for any of the detected pathogens.

Notes:

Oral Presentations ESVP/ECVP

035 *MERS CORONAVIRUS* INFECTION OF RABBITS

Notes:

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Introduction: A new coronavirus (CoV), Middle East respiratory syndrome CoV (MERS-CoV), is causing an ongoing outbreak in humans, sometimes resulting in severe or even fatal pneumonia. MERS-CoV uses dipeptidyl peptidase 4 (DPP4) as a functional receptor and is able to infect cells of a limited number of animal species including bats, camels, goat and (non-)human primates *in vitro* and so far there is no good animal model for human disease. Because the virus binding region in rabbit DPP4 closely resembles that in human DPP4, we tested whether rabbits can be infected with MERS-CoV as an animal model for MERS-CoV infection in humans.

Materials and Methods: Sixteen rabbits, serologically negative for MERS-CoV, were inoculated with MERS-CoV or sham inoculum via nose and trachea and swabs were taken frequently. The rabbits were euthanised on 3, 4 or 21 days ($n = 4$ per day) after inoculation and during necropsy samples were taken for pathology, immunohistochemistry, in situ hybridisation and virology.

Results: The rabbits demonstrated no clinical signs and on 3 and 4 days after inoculation the rabbits had high viral loads as determined by PCR and scattered virus antigen expression in the lungs and nose associated with mild alveolitis and moderate rhinitis.

Discussion: Our study demonstrates that rabbits can be infected with MERS-CoV with virus replication in the lungs and nose. Therefore, rabbits infected with MERS-CoV may be used as a model to study the pathogenesis of MERS, transmission of MERS-CoV and to test intervention strategies aimed at inhibition of MERS-CoV replication *in vivo*.

036 NO EVIDENCE OF *SARCOCYSTIS CALCHASI* INVOLVEMENT IN MENINGOENCEPHALITIS OF UNKNOWN ORIGIN IN MAMMALS

Notes:

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Introduction: *Sarcocystis calchasi* is an intracellular, protozoan parasite belonging to the phylum Apicomplexa. It has been identified as the causative agent of Pigeon Protozoal Encephalitis (PPE) during an outbreak in Berlin in 2008. PPE is an ongoing threat as new cases are continuously diagnosed in pigeons in the Berlin area. Birds and mammals may usually serve as intermediate hosts of other *Sarcocystis* spp., thus a retrospective study was conducted to determine whether *S. calchasi* may be involved in cases of meningoencephalitis of unknown origin (MUO) in mammals.

Materials and Methods: Formalin fixed, paraffin embedded (FFPE) samples of 142 brains with MUO of different mammalian species (dog, cat, pig, cattle, sheep, guinea pig, horse, goat, mouse, raccoon, ferret, hamster, mink, mane wolf) from between 1989 and 2012 were reexamined histologically using HE stain. DNA was isolated from FFPE material and screened by PCR with primers specific for the *18S rRNA* and the *ITS-1* gene to detect *S. calchasi* or other apicomplexan parasites, respectively.

Results: In all samples the diagnosis of non-suppurative (lymphoplasmacytic and/ or granulomatous) meningoencephalitis was confirmed but no parasitic structures were found. DNA of *S. calchasi* or other apicomplexan parasites could not be detected in any of the samples.

Discussion: Despite seemingly high prevalence of PPE and persistent threat of *S. calchasi* in pigeons in the Berlin area, no evidence was found for a role of this parasite in MUO in mammalian species.

Oral Presentations ESVP/ECVP

037 *MYCOBACTERIUM TUBERCULOSIS* INFECTION IN CAPTIVE WHITE RHINOCEROS (*CERATOTHERIUM SIMUM SIMUM*)

Notes:

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Introduction: An outbreak of *Mycobacterium tuberculosis* affected several species in a Swedish zoo. Three white rhinoceroses (*Ceratotherium simum simum*) tested positive inconsistently to serology and tuberculin skin tests. *M. tuberculosis* was cultured once from a nasal wash from one of the rhinoceros.

Materials and Methods: Necropsy, histopathology and real-time PCR on formalin-fixed, paraffin-embedded tissues were conducted.

Results: At necropsy, two rhinoceros had a nodule (0.4 cm and 1 cm) each in the lungs. These were clusters of solid granulomas, formed by epithelioid macrophages, multinucleated giant cells, lymphocytes, fibrosis and neovascularisation. Initially, no AFB could be detected histologically and tuberculosis could not be confirmed by culture from numerous tissues. Retrospectively, in-depth studies were done. A few AFB were observed on serial sections of the granulomas and *M. tuberculosis complex* infection was confirmed in the 2 rhinoceros by real-time PCR.

Discussion: Post-mortem confirmation of tuberculosis, in particular in exotic species, can be a challenge. No visible lesion tuberculosis (or minimal lesion, as in this case) is a frequent presentation of infected but clinically healthy animals. A thorough systematic necropsy, including thin slicing of lungs and lymph nodes increases the likelihood of detecting small lesions, which is the key for the confirmation of tuberculosis. A further difficulty is that the histomorphology of the lesions differs in different taxonomic groups. This study describes the pathology of minimal-lesions tuberculosis in rhinos, presents post mortem tools and discusses diagnostic challenges. A successful pathology-based confirmation of tuberculosis is of particular importance on valuable individuals euthanised due to positive reactivity to live tests.

038 *CHLAMYDIA PNEUMONIAE* IN SNAKES – A CASE STUDY

Notes:

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Introduction: Chlamydial infections in reptiles and amphibians have been associated with granulomatous inflammation in the inner organs. *Chlamydia (C.) pneumoniae* has been repeatedly detected in these lesions. However, limited knowledge is available on the clinical significance of chlamydial infections in snakes.

Materials and Methods: Mortality cases (n=6) during the winter rest (2010-2013) were reported in a captive snake population. Dead snakes were investigated by histopathology and molecular tests for *Chlamydiaceae*. The total remaining population (n=47) was tested for *Chlamydiaceae* by PCR on swabs (choana, cloaca). *C. pneumoniae*-positive animals (n=7) were treated with Marbofloxacine and chlamydial shedding was monitored by PCR. Concurrent viral infections (adenoviruses, paramyxoviruses) were tested on swab samples.

Results: Infection with *C. pneumoniae* was confirmed in a horned viper (*Vipera ammodytes*) with histiocytic granulomas in heart and liver. The other mortality cases were negative for *Chlamydia*. By PCR, seven out of 47 snakes were positive for *C. pneumoniae*. All positive snakes were either horned or Caucasus vipers *(Vipera kaznakovi)*, were clinically unremarkable and were positive either in the choana or the cloaca or both. Marbofloxacine treatment was associated with negative swab samples after five to ten days of treatment in five snakes. One snake remained positive up to 4 months after initial testing. Investigations for viral agents were negative (n=47).

Discussion: *C. pneumoniae* has been detected in granulomas in reptiles but might also represent an opportunistic pathogen in snakes. Chlamydial shedding via the choana and the cloaca or both is likely to be the source of infection.

Oral Presentations ESVP/ECVP

039 *CHLAMYDIALES* IN THE KOALA (*PHASCOLARCTOS CINEREUS*) – ORGAN DISTRIBUTION AND RELATED HISTOPATHOLOGICAL FINDINGS

Notes:

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Introduction: Chlamydial infections in koalas can cause chronic diseases leading to blindness and sterility. However, knowledge about the systemic spread of chlamydiae in the inner organs of the koala and related pathological organ lesions is limited.

Materials and Methods: In this study, a thorough investigation of organs from 23 koalas was performed and their histopathological lesions were correlated to molecular chlamydial detection. To reach this goal, 246 formalin-fixed and paraffin embedded organ samples from 23 koalas were investigated by histopathology, *Chlamydiaceae* real-time PCR and immunohistochemistry (IHC), ArrayTube Microarray for *Chlamydiaceae* species identification as well as *Chlamydiales* real-time PCR and sequencing.

Results: By PCR, two koalas were positive for *Chlamydia pecorum*. By IHC, *Chlamydiaceae* were detected in 10 tissues out of nine koalas. The majority of these (n=6) had positive labeling in the urogenital tract related to histopathological lesions such as cystitis, endometritis, pyelonephritis and prostatitis. Somehow unexpected was the positive IHC labeling in the gastrointestinal tract including the cloaca. Immunoreactivity in lung and spleen indicated systemic spread of infection. Uncultured *Chlamydiales* were detected in several organs of seven koalas by PCR, and four of these suffered from plasmacytic enteritis of unknown aetiology.

Discussion: *Chlamydiales* might be associated with plasmacytic enteritis. *Chlamydiaceae* can cause systemic infections but can be also detected in the intestine without association to histopathological lesions. The gastrointestinal tract might play a role being the site for persistent chlamydial infections and being a source for reinfection of the genital tract as recently shown in a mouse model.

040 HIGH INCIDENCE OF NEOPLASIA IN A GROUP OF CAPTIVE RICHARDSON'S GROUND SQUIRRELS (*UROCITELLUS RICHARDSONII*)

Notes:

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Introduction: Richardson's ground squirrels (RGS) are infrequently kept exotic pets and there is a lack of pathological studies on captive populations. Cancer is regarded uncommon but hepadnavirus-associated hepatocellular carcinoma has been documented in a number of individuals. This study demonstrates the high incidence of neoplasia in a group of rescue animals (n=13).

Materials and Methods: From February 2011 to May 2014 nine animals were examined utilising combinations of radiography/computer tomography, incisional/excisional biopsies and post mortem examination. Histology (n=6) and transmission electron microscopy (TEM) (n=1) was carried out to investigate the cause of death and presence of a viral agent.

Results: Identified neoplasms comprised of hepatocellular carcinoma (n=3), lipoma (n=3), elodontoma (n=3), leiomyosarcoma (n=1), hepatic adenoma (n=1), squamous cell carcinoma (SCC) (n=1), myelolipoma (n=1) and renal papillary adenocarcinoma (RPA) (n=1). The mean age at diagnosis ranged from 25 to 47 months of age. Seven females and three males were affected. Elodontoma was the only tumour diagnosed in males. Mean survival was 1 to 14 months. TEM could not identify virus particles.

Discussion (and/or Conclusions): Contrary to previous findings this study indicates a high incidence of neoplasia in RGSs and includes the first reports of elodontoma, leimyosarcoma, SCC, myelolipoma and RPA. The association of hepatic carcinomas with hepadnavirus was not confirmed by TEM but further investigations are necessary. To our knowledge, animals are not related but detailed pedigree information is unavailable and a genetic component possible. Veterinarians should be aware of the high tumour incidence and screening is recommended for early detection.

Oral Presentations ESVP/ECVP

O41 CALPOX VIRUS MARMOSET MODEL- A NEW PRIMATE ANIMAL MODEL FOR ORTHOPOXVIRUS INFECTIONS

Notes:

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Introduction: Smallpox is widely regarded as potential threat used in bio terrorist attacks but zoonotic orthopoxviruses induce outbreaks with severe pathology as well. New vaccines and therapeutics against OPV infections are urgently needed and have to be tested in animal models.

Material and Methods: We here introduce a new promising animal model which is based on the experimental infection of marmosets (*Callithrix jacchus*) with the calpox virus. The calpox virus, a cowpox like virus, was discovered during an outbreak of an atypical cowpox infection among a group of new world monkeys living in a private husbandry. During this poxvirus outbreak 30 from 80 animals died.

Results: The disease was strongly reproducible under experimental conditions. Marmosets infected intranasally developed similar reproducible symptoms and died within two to three days after onset of the first symptoms, even at low infectious doses. Using different infectious doses of calpox virus the 50 % monkey infectious dose (MID50) was calculated with $8.3x10^2$ pfu showing that calpox virus is highly pathogenic for marmosets.

Conclusions: Advantages over other animal models are the good reproduction of the syndrome, the extremely low infectious dose and the choice for intranasal application mimicking a true natural route. The less human pathogenicity, the small animal size and the easy handling of New World monkeys compared to Old World monkeys makes this animal model attractive for vaccination and therapeutic studies as shown exemplary on a vaccination study using two vaccines, – modified vaccinia virus Ankara (MVA) and Vaccinia virus Lister-Elstree (VACV LE-BN).

O42 EVIDENCE OF VERTICAL TRANSMISSION OF BOID INCLUSION BODY DISEASE ARENAVIRUSES

Notes:

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Introduction: Boid inclusion body disease (BIBD) is an often fatal disease that has been observed worldwide in captive tropical and subtropical boid snakes. Recently, arenaviruses have been identified as the causative agent. So far, it is uncertain whether boids are primary or paratenic hosts of the viruses, and the transmission routes are not known. However, BIBD can rapidly spread horizontally between animals, and it is assumed that the viruses are transmitted vertically (transovarially) in ovoviviparous boids, like *Boa constrictor*.

Materials and Methods: An investigation into a possible vertical transmission was carried out on the offspring of 3 BIBD positive *B. constrictor* breeding pairs from a Swiss boid collection, i.e. 17 juveniles (clutch 1, individually housed), 3 perinates (clutch 2), and 6 embryos (clutch 3). Blood cytology, tissue histology and immunohistology for viral antigen, PCR and boid tissue culture inoculation were employed.

Results: Apart from the adult animals, 15/17 juveniles tested positive and exhibited the characteristic eosinophilic intracytoplasmic inclusion bodies in almost all cell types, including spermatozoa, oocytes, and salpinx. One of the 3 perinates was clearly positive. In one perinate and the 6 embryos, the morphological results were questionable, and the in vitro inoculation studies are not yet completed.

Conclusions: The study provides strong evidence of vertical transmission of BIBD in *B. constrictor*. So far, the mode and time period of infection during the embryonal and fetal development is not clear, since cells in the reproductive tract of both sexes carry the virus.

Oral Presentations ESVP/ECVP

O43 INDUCTION OF TRACHEAL ANTIMICROBIAL PEPTIDE EXPRESSION FOR PREVENTION OF BACTERIAL PNEUMONIA IN CATTLE

Notes:

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Introduction: Bacterial pneumonia results from complex interactions among host, pathogens, and the environment, and can be considered to result from failure of the respiratory defences against these pathogens. This presentation summarises our recent work on the role of tracheal antimicrobial peptide (TAP)—a beta-defensin of cattle important in innate immunity—in pathogenesis of pneumonia in cattle, and in development of novel methods to stimulate this innate immune response for prevention of pneumonia in cattle.

Results: TAP is shown to have bactericidal activity against the pathogens that cause pneumonia in cattle, with no evidence that these host-adapted pathogens have evolved resistance to this peptides. Genetic polymorphisms were identified that affect function and expression of this peptide, suggesting the potential for genetic improvement of disease resistance. Importantly, well-recognised risk factors for development of bacterial pneumonia in cattle, including corticosteroids (as a model of stress) and BVD viral infection, reduced the inducible expression of tracheal antimicrobial peptide, suggesting this as one mechanism of susceptibility to pneumonia. The effect of various agonists of cell surface receptors on induction of tracheal antimicrobial gene expression was investigated. In addition to LPS, agonists of TLR 1/2 and interleukin-17AR induced gene expression via an NF-kB-dependent mechanism.

Materials and Methods: TAP gene expression was measured by quantitative RT-PCR in cultured bovine tracheal epithelial cells following exposure to various agonists, corticosteroids, and signalling pathway inhibitors. Antimicrobial effects of synthetic TAP were studied in vitro against isolates of *Mannheimia* and other pathogens.

Conclusions: These findings imply that suppression of beta-defensin expression contributes to development of bacterial pneumonia in stressed or virus-infected cattle. Thus, signalling pathways including LPS, TLR1/2 and IL-17A are targets for developing methods to stimulate innate immune responses in the lung of cattle during periods of risk for bacterial pneumonia.

O44 EFFECT OF *CLOSTRIDIUM PERFRINGENS* BETA-TOXIN ON THE PORCINE SMALL INTESTINAL MUCOSA

Notes:

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Introduction: Clostridium perfringens beta-toxin (CPB) is the essential virulence factor of *C. perfringens* type C strains which cause fatal necrotising enteritis in pigs, humans and other mammalian species. Although the importance of CPB in the pathogenesis of this disease has been demonstrated, its natural target cells and molecular mode of action have not been studied in detail. We identified small intestinal endothelial cells as potential natural target cells, whereas other studies indicated that small intestinal epithelial cells are the major target. We hypothesised that primary epithelial damage in the small intestine leads to CPB penetration into the tissue, binding of CPB to endothelial cells and subsequent vascular damage.

Materials and Methods: We investigated the effect of CPB on the small intestinal epithelium using two parallel approaches: 1. Exposure of a porcine jejunal epithelial cell line 2. Exposure of porcine neonatal jejunal mucosal explants to recombinant CPB and *C. perfringens* type C culture supernatants. We localised CPB by immunohistochemistry in explants (n= 205) and performed trans-epithelial-resistance measurements in polarised epithelial cell layers (n= 94).

Results: Small intestinal epithelial cells were not affected by CPB. In porcine small intestinal mucosal explants, CPB bound to endothelial but not epithelial cells. Damage of the epithelium or the *tunica muscularis* enabled CPB to bind more rapidly to endothelial cells.

Discussion (and/or Conclusions): In conclusion, our results suggest that endothelial cells are the primary target of CPB in the small intestine. The epithelial damage, required for penetration of the toxin into the tissue, is most likely induced by additional factors.

Oral Presentations ESVP/ECVP

O45 INTRACEREBRAL SPREAD OF *LISTERIA MONOCYTOGENES* IN RUMINANTS OCCURS BY INTRA-AXONAL MOTILITY

Notes:

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Introduction: *Listeria monocytogenes* (LM) rhombencephalitis is a rapidly progressive and frequently fatal disease of ruminants. In view of the observed ability of LM to spread within cranial nerves, we hypothesised that the disease further progresses within the central nervous system by intra-axonal spread.

Materials and Methods: Neuroanatomical mapping of lesions, immunofluorescence and electron microscopy for cellular localisation of LM were performed in ruminants with naturally occurring rhombencephalitis. To consolidate the *in vivo* data, bovine fetal brain cell cultures were experimentally infected with a GFP expressing LM strain isolated from encephalitis and life-cell-imaging was performed.

Results: Mapping of lesions revealed a consistent pattern with preferential affection of certain white matter tracts in the rostral areas. In immunofluorescence, LM was predominantly present within phagocytes of microabscesses, but in unaffected areas LM were found closely associated with neurofilaments. Ultrastructurally, LM surrounded by networks of fibrillary structures consistent with actin tails were observed inside axons. Various stages of axonal degeneration occurred, which were associated with intra-axonal invasion of neutrophils. The latter frequently contained LM. In bovine brain cell cultures, bacteria were moving over considerable distances within axon-like processes.

Discussion (and/or Conclusions): There is convincing evidence for intra-axonal spread of LM within the brain, which triggers neutrophil invasion into the axonal space providing a new replication site for LM. The latter may contribute to the infection of new axons. Both the intra-axonal spread of LM and the intrathecal immune response may, therefore, play an important role in the progression of listeric rhombencephalitis.

O46 DECIPHERING THE MOLECULAR PATHOLOGY OF POXVIRUSES USING A LOSS-OF-FUNCTION SI-RNA-BASED SCREEN

Notes:

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Introduction: Poxviruses are important pathogens of humans and animals. They undertake a complex replication cycle within the cytoplasm of a host cell involving multiple interactions with many cellular pathways and processes.

Materials and Methods: In order to examine the molecular interactions between poxviruses and the host cell we undertook a high throughput loss-of-function screen of *Vaccinia virus* (VACV), the prototype poxvirus. Cellular protein levels in HeLa cells were knocked down using siRNA prior to infection with a fluorescently tagged VACV strain. Viral replication was assessed by measuring fluorescence.

Results: A "hit list" of strongly pro-viral and anti-viral cellular proteins was compiled and detailed investigations carried out into a selection of the most interesting and novel hits in order to pinpoint their role in the VACV replication cycle. The cell signalling protein TRAF2 was identified as a facilitator of virus entry, and the small GTPase Rab1a was discovered to be required for effective intercellular spread of VACV.

Conclusion: This unbiased loss-of-function screen of VACV identified novel host factors important for virus replication, revealing new details of viral molecular pathology and providing new targets for anti-viral therapeutics.

Oral Presentations ESVP/ECVP

047 LIVER CHANGES IN SHEEP VACCINATED AND NON VACCINATED WITH RECOMBINANT CATHEPSIN L1 AND CHALLENGED WITH *FASCIOLA HEPATICA*

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Introduction: *Fasciola hepatica* is a helminth parasite responsible for significant economic losses in livestock worldwide. There is an increasing interest in developing protective vaccines for ruminants. The aim of this work was to evaluate hepatic lesions in sheep immunised with recombinant cathepsin L1 and in infected controls.

Materials and Methods: Thirty-seven sheep were divided into: group 1 (n = 14) immunised with recombinant cathepsin L1 (CL1); group 2 (n = 12) immunised only with adjuvant montanide , and group 3 (n = 9), not immunised. Animals were orally infected with 5 weekly dose of 30 metacercariae of *F. hepatica*. Sheep were euthanised at week 19 post-infection. Fluke burdens and histopathological evaluation of liver samples was performed.

Results and Discussion: No significant difference in the number of parasites among the three groups was found. The three groups showed typical lesions of chronic fascioliosis with bile duct hyperplasia, portal fibrosis, infiltration of lymphocytes and plasma cells, fibrotic infiltration routes with haemosiderin-laden macrophages, lymphocytes and plasma cells, granulomas with cellular debris surrounded by multinucleated giant cells, lymphocytes, macrophages and plasma cells. Eosinophil infiltration was abundant in most animals and globular variable infiltrated leukocytes. Chronic tracts were significantly lower in the group immunised with CL1, which suggests that the vaccine could interfere with the role of hepatic tissue digestion by this protease, although this mechanism does not induce death of significant number of parasites.

Notes:

048 ENTEROMYXOSIS EXPOSED: RNA-SEQ ANALYSIS SHEDS LIGHT ON PATHOGENETIC MECHANISMS AND HOST IMMUNE RESPONSE IN A FISH-PARASITE MODEL

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Introduction: Enteromyxosis caused by the intestinal myxozoan parasite *Enteromyxum scophthalmi* is a serious threat for turbot (*Scophthalmus maximus*, L.) aquaculture, causing severe catarrhal enteritis leading to a cachectic syndrome, with no therapeutic options available. The study of host-parasite interaction and disease pathogenesis still present many aspects to be disclosed, nevertheless, to date there is a gap in the analysis of transcriptomic changes induced by *E. scophthalmi* in turbot organs.

Materials and Methods: After a histopathological evaluation of samples, RNA-seq technology was applied to head kidney, spleen and pyloric caeca of three severe infected turbot and their respective control.

Results: A huge amount of information was generated, finding 4,762 differently expressed genes between control and infected fish. Associated gene functions were studied on the basis of gene ontology terms and available literature, then the most interesting DE genes were categorised in: 1) immune and defence response; 2) apoptosis and cell proliferation; 3) iron and erythropoiesis; 4) cytoskeleton and extracellular matrix and 5) metabolism and digestive function.

Discussion (and/or Conclusions): This is the first application of RNA-seq technology to the study of a fish-parasite model. The results have generated novel hypotheses regarding the role of the components bridging innate and adaptive immune response together with increasing evidence of inadequate adaptive immunity and exacerbated local immune response. Also, the transcriptomic analysis has revealed details on the genetic basis underlying the enteromyxosis-associated clinical signs and lesions. This knowledge is essential to investigate the disease pathogenesis, identify resistance-related genes and devise effective preventive and therapeutic strategies.

Notes:

Oral Presentations ESVP/ECVP

049 WIDESPREAD GLOMERULAR COLLAGEN TYPE V ACCUMULATIONS AND HEPATIC PERISINUSOIDAL FIBROSIS IN CANINE CASES OF COLLAGEN TYPE III GLOMERULOPATHY

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Introduction: Collagen type III glomerulopathy (Col3GP) is a rare renal disease characterised by massive glomerular accumulations of collagen type III. The disease occurs in both humans and animals, and in dogs an autosomal recessive inheritance pattern has been shown. The pathogenesis is unknown. In the present study we describe a Drever dog litter affected by an early onset version of this glomerular disease.

Materials and Methods: Four of nine Drever puppies developed renal failure within 50 days of age, and were euthanised due to bad prognosis. Kidney autopsy specimens were studied by light microscopy, transmission electron microscopy, conventional immunofluorescence and specific immunohistochemical analysis for collagens I, III and V. Liver specimens were studied by light microscopy, transmission electron microscopy and immunohistochemical analysis for collagen III.

Results: Characteristic morphological findings of Col3GP were present in all affected cases, including membranoproliferative pattern injury and massive glomerular collagen type III deposition. Further morphological characterisation demonstrated that the deposited glomerular collagen was composed of a mixture of collagens III and V. The distribution of collagen V corresponded to the localisation of collagen III, however, differences in staining intensity displayed that collagen type III is the dominating component. Mild hepatic perisinusoidal fibrosis was present in all affected cases.

Conclusions: This is the first report documenting concurrent hepatic fibrosis and glomerular accumulations of collagen type V in canine cases of Col3GP. Liver involvement suggests the possibility that canine Col3GP might be a systemic disease.

Notes:

050 BOVINE ZINC DEFICIENCY-LIKE SYNDROME: PATHOLOGICAL AND CLINICAL ALTERATIONS IN CALVES WITH A NONSENSE MUTATION IN *PLD4*

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Introduction: Recently, calves with the phenotype of bovine hereditary zinc deficiency (BHZD) have been reported in the Fleckvieh population. BHZD-calves display dyskeratotic skin lesions, runting and respiratory and digestive tract infections as a result of compromised immune function. *SLC39A4*, the gene responsible for BHZD was excluded as underlying gene in the reported calves. Instead, a causative nonsense mutation in a phospholipase D4 encoding gene (*PLD4*) was identified. The objective of this study was the phenotypic characterisation of this newly identified disorder.

Material and Methods: Eight calves with a history of chronic dermatitis were submitted to the Clinic for Ruminants between 5 and 19 weeks of age. Due to severely disturbed general condition, six calves were euthanised few days after submission. Two calves were clinically examined over several weeks before euthanasia. Tissues taken at necropsy were processed and stained according to routine protocols for histologic and electron microscopic examinations.

Results: Severe dermatitis, hyperplasia of lymph nodes, enteritis and respiratory tract infections were the most prevalent findings. Some calves displayed ulcerations of the oral mucosa. Laboratory findings included anaemia, leukocytosis, hypalbuminaemia and zinc concentrations within or above reference ranges. The histologic examination of the skin revealed severe hyper- and parakeratosis, oedema and necrosis of the epidermis, and dermal and epidermal infiltration of numerous inflammatory cells.

Discussion/Conclusions: The phenotype of zinc deficiency-like syndrome resembles BHZD. However, zinc substitution did not ameliorate clinical signs, intestinal Paneth cells displayed no inclusions and thymus weights did not indicate hypoplasia, therefore separating this disorder from BHZD.

Notes:

Oral Presentations ESVP/ECVP

O51 IMMUNOHISTOCHEMICAL CHARACTERISATION OF A CUTANEOUS PIGMENTED NEUROFIBROMA IN A PIG

Notes:

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Introduction: Tumours consisting of melanocytic and neural components are diagnostic challenging and provoke questions regarding their histogenesis. Neurofibromas are benign peripheral nerve sheath tumours (PNST) arising from Schwann cells (SCs) and a mixture of perineurial cells and fibroblasts. Pigmented variants are exceedingly rare in humans. The present report describes the histological and immunohistological characterisation of a cutaneous PNST in a pig sharing features of pigmented neurofibroma in man.

Materials and Methods: A young female pig submitted for necropsy. Tissues were routinely processed for histology and immunohistochemistry using antibodies against S100, GFAP, laminin, periaxin, Sox2, p75NTR, vimentin, SMA, Melan A, and cytokeratin.

Results: Grossly, the only lesion consisted of a poorly demarcated, non-encapsulated and focally pigmented mass localised in the subcutis of the right abdominal wall. Microscopically, spindle-shaped neoplastic cells were arranged in nodules of different sizes and in short fascicles separated by extensive fibrous tissue. Focal accumulation of elongated cells containing melanin pigment was observed. Neoplastic cells within nodular formations were immunoreactive to S100, laminin, Sox2, $p75^{NTR}$ and rarely GFAP and vimentin. Cells resembling perineurial cells expressed $p75^{NTR}$, cytokeratin and SMA. Vimentin and SMA were found in the fibrotic component. About 2% of the cells expressed Melan A. Immunolabeling for periaxin was negative.

Conclusion: These findings are compatible with a neurofibroma with melanocytic differentiation. Expression of $p75^{NTR}$ and Sox2 in neoplastic cells may suggest SCs precursors or immature SCs as cells of origin. The presence of a melanocytic component may indicate melanocytes arising directly from SCs precursors, as described for the developing nerves. Moreover, $p75^{NTR}$, SMA and cytokeratin immunoreactivities in perineurial cells may indicate a myoepithelial cell lineage in pigs.

O52 COMPARISON OF THORACIC AORTIC BIOMECHANICAL PROPERTIES IN FRIESIAN AND WARMBLOOD HORSES

Notes:

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Introduction: Thoracic aortic rupture is extremely rare in horses. The Friesian horse however shows a breed predilection. Considering the consistent location of the rupture near the scar of the ligamentum arteriosum Botalli and the high inbreeding rate, a genetic condition is likely. The aim of this study was to compare the biomechanical properties of the thoracic aorta between Friesian and Warmblood horses.

Materials and methods: 3 groups of horses were sampled: affected Friesian horses (n=5), healthy Friesian horses (n=7) and Warmblood horses (n=7). Aortic strips were obtained in the axial direction at 3 locations: the level of the ligamentum arteriosum outside the lesion (B), mid thoracic aorta (T1) and distal thoracic aorta (T2). Rectangular samples were used for uniaxial tensile testing and dogbone-shaped samples for tearing tests. Linearised stiffness, failure stress and viscoelasticity were evaluated.

Results: The measured mechanical values showed no significant interaction with the group of horse ($p>0,05$). There were however significant differences between the sample locations ($p<0.05$). The distal site of the thoracic aorta (T2) could withstand a higher maximum stress and was less compliant compared to the proximal sites (B and T1). The aorta at the ligamentum arteriosum was less visco-elastic compared to T1 and T2.

Conclusion: Regional mechanical differences were found over the length of the equine thoracic aorta. No significant mechanical differences were found between the different horse breeds. This suggests the existence of a local, hereditary defect at the level of the ligamentum arteriosum Botalli in the affected Friesian horses.

Oral Presentations ESVP/ECVP

O53 CONCOMITANT CUTANEOUS NEOSPOROSIS AND TOXOPLASMOSIS IN A GOLDEN RETRIEVER

Notes:

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Introduction: This report describes a case of concomitant cutaneous neosporosis (CN) and cutaneous toxoplasmosis (CT) in a dog. .

Materials and Methods: A 10 years old, intact female Golden Retriever under treatment with cyclosporine for an autoimmune disorder had sudden development of multifocal cutaneous nodules. Imprints, fine-needle aspirates and skin punch biopsies were submitted. Immunofluorescence antibody test (IFAT) on serum samples was performed twice at a one month interval. Unstained smears and deparaffinised sections of skin were immunochemically stained with polyclonal anti-*Toxoplasma gondii (TG)* and anti-*Neospora caninum (NC)* primary antibodies. PCR assay with pimers for *TG* and *NC* were conducted using DNA extracted from cutaneous formalin-fixed, paraffin-embedded tissue.

Results: Cytology demonstrated a prevalence of degenerated neutrophils admixed with fewer reactive macrophages containing numerous intracytoplasmic crescent shaped, 4-6 µm microrganisms, with a light basophilic cytoplasm and a central nucleous (tachyzoites). Histology revealed diffuse and severe neutrophilic, histiocytic, and eosinophilic dermatitis and panniculitis associated with necrotising vasculitis. Elevated numbers of free and cytoplasmic tachyzoites within macrophages and epidermal and follicular keratinocytes were present. IFAT was positive for both *TG* and *NC*, with increased *TG* antibody titers after one month. Immunochemistry and PCR confirmed a concomitant *TG* and *NC* cutaneous infection. Clindamycin administration (11 mg/kg PO every 12 hours) and withdrawal of immunosuppressive medication resulted in clinical remission.

Discussion (and/or Conclusions): CN and CT are rare manifestation of both diseases. To the best of our knowledge this is the first report of a simultaneous infection of *TG* and *NC* with cutaneous anatomical location.

O54 Streptococcal aortic valve endocarditis in a Siamese cat

Notes:

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Introduction: Infective endocarditis is an extremely rare heart disease in cats. A 10 years old spayed male Siamese cat suddenly died. The cat had been treated with a daily dose of 2.5 mg cortisone the last 7 years for an allergy, probably against dust mites.

Macroscopical findings: The cat was in good body condition. He had only two canine teeth left in the upper jaw, which showed moderate to severe cremor dentis. The rest of the teeth were removed due to Feline Odontoclastic Resorptive Lesions (FORL). On the aortic valves there were yellowish cauliflower-like vegetations. The lungs were congested, and the right kidney had scattered small indentations.

Microscopical findings: The aortic valves were covered with debris, rich in Gram-positive coccoid bacteria, over a proliferation of fibrous tissue and inflammatory cells, neutrophils and lymphocytes. In the myocardium there were scattered multiple small foci with neutrophils and macrophages, as well as many bacterial colonies within vessels without inflammatory reaction. The kidneys showed moderate multifocal chronic inflammation. In addition there were several bacterial colonies with no reaction. In the spleen there were small foci with degenerate neutrophils and macrophages. Bacterial culture of the aortic valve showed growth of *Streptococcus* sp.

Discussion: The earlier episodes of FORL, and the prednisolone treatment, probably acted as predisposing factors for the streptococcal infection, which eventually spread to the aortic valves and the myocardium.

Oral Presentations ESVP/ECVP

O55 BIOKINETICS AND INHALATION TOXICITY OF CERIUM DIOXIDE AND BARIUM SULFATE NANOPARTICLES AFTER 1, 4, 13 AND 52 WEEKS OF EXPOSURE

Notes:

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Introduction: Comprehensive data on the carcinogenicity of air-borne nanoparticles are lacking. CeO_2 and $BaSO_4$ are currently being tested in a chronic and carcinogenicity inhalation study (OECD TG 453), within the EU-project NanoREG.

Materials and Methods: Rats were exposed to 0.1, 0.3, 1 and 3mg/m³ CeO_2 and 50mg/m³ $BaSO_4$ for 6 h/d for 2 years. Interim results after 13 and 52 weeks and results of previous studies with one and four weeks of exposure to 0.5, 5 and 25mg/m³ CeO_2 are available. Examinations included burdens and histopathology of the respiratory tract and bronchoalveolar lavage fluid (BALF) analysis.

Results: Inhaled CeO_2 is deposited in the lung and cleared with a half-time of 40 days; the clearance was retarded by exposure to higher concentrations (25mg/m³). Lung burden after 13 weeks of exposure to 3mg/m³ were 1.4mg/lung. Pulmonary inflammation was observed by neutrophils in BALF after 1 and 4 weeks of exposure to 5 and 25mg/m³ and after 13 weeks of exposure to 1 and 3mg/m³ CeO_2. The dose-response curve's slope was steeper after shorter exposure times. Granulomatous inflammation was observed by histopathology after 4 weeks exposure to 5 and 25mg/m³ CeO_2. $BaSO_4$ is rapidly cleared from the lung ($t_{1/2}$ 7 days); lung burdens were 1.7mg/lung after 13 weeks exposure. Neutrophils in BALF were not increased after 4, but slightly increased after 13 weeks.

Conclusions: The time course of particle burdens and effects in the lung are described. A link between retarded clearance and inflammation in the lung driven by neutrophils and macrophage and subsequent granulomatous inflammation was observed.

O56 INHALED MULTI-WALLED CARBON NANOTUBES MODULATE THE IMMUNE RESPONSE OF TRIMELLITIC ANHYDRIDE-INDUCED CHEMICAL RESPIRATORY ALLERGY IN BROWN-NORWAY RATS

Notes:

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Introduction: The interactions between exposure to nanomaterials and existing inflammatory conditions have not been fully established.

Materials and Methods: Multi-walled carbon nanotubes (MWCNT; Nanocyl NC 7000 CAS no. 7782-42-5; count median diameter in atmosphere 61 ± 5 nm) were tested by inhalation in high IgE-responding Brown Norway (BN) rats with trimellitic anhydride (TMA)-induced respiratory allergy. The rats were exposed 2 days/week over a 3.5-week period to a low (11 mg/m3) or a high (22 mg/m3) concentration of MWCNT. Non-allergic animals exposed to MWCNT, and unexposed allergic and non-allergic rats served as controls. At the end of the exposure period, the allergic animals were re-challenged with TMA.

Results: Breathing pattern of rats, allergic to TMA, did not change by the inhalation of MWCNT. Histopathological examination of the respiratory tract showed agglomerated- aggregated MWCNT in the lungs and in the lung-draining lymph nodes. Frustrated phagocytosis was observed as incomplete uptake of MWCNT by the alveolar macrophages and by clustering of cells around MWCNT. Large MWCNT agglomerates/aggregates were found in granulomas in the allergic rats, suggesting decreased macrophage clearance in allergic rats. In allergic rats, MWCNT exposure decreased serum IgE levels and the number of lymphocytes in broncho-alveolar lavage. MWCNT in the alveoli presented as clusters with a relatively high content of iron (scanning electron microscopy combined with element analysis or SEM/EDX).

Conclusion: MWCNT did not aggravate the acute allergic reaction, but modulated the allergy-associated immune response.

Oral Presentations ESVP/ECVP

057 FREE FLOATING BRAIN SECTIONS FOR IMMUNOFLUORESCENCE MARKERS: A TECHNICAL AND SCIENTIFIC APPROACH

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Introduction: Production of "free floating sections" is regarded as a new method that can be used for immunofluorescence staining. This method is clearly the best way to go for optimal antibody penetration and even staining of thick sections which later on can be used for a confocal microscopical analysis. This presentation covers the technical work pattern of the method starting with the tissue preparation and conservation, through brain accurate dissection, staining and tissue evaluation.

Materials and methods: The technical aspects such as tissue fixation and tissue preparations, brain sectioning, staining protocols will be described.

Results: Multiple examples of case studies are presented of projects that had combined the method such as stroke and Parkinson studies in laboratory animal models that were performed in Pharmaseed laboratories.

Discussion: Finally a discussion will be held where the advantages of the current method will be pointed out compared to the classical immunohistochemistry methods.

Notes:

058 HALO™-NEXT GENERATION IMAGE ANALYSIS FOR QUANTITATION OF INTERSTITIAL NEPHRITIS IN PIGEONS AFTER EXPERIMENTAL INFECTION WITH *PIGEON PARAMYXOVIRUS-1*

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Introduction: *Pigeon paramyxovirus-1* (PPMV-1) summarises specific subtype-1 avian paramyxoviruses that are endemic in pigeon populations worldwide. Clinical signs of acute disease in pigeons include polyuria and polydipsia. Histopathology reveals renal tubular degeneration and necrosis, followed by mononuclear infiltration in various extend. Aim of the study was to apply image analysis using the HALO™ system to quantify the interstitial nephritis as sequela of experimental PPMV-1 infection.

Materials and Methods: 5 pigeons were infected with 10^5 $TCID_{50}$ of PPMV-1. As sentinels, 2 pigeons were added at day 2 post infection (p.i.). As non-infected-controls served 3 pigeons from the same breeder. After 21 days p.i. all animals were euthanised and submitted for necropsy and further virological and histopathological investigation.

Results: At day 5 p.i. one pigeon showed paralysis of the wings and died at day 7 p.i. All other animals survived, with clinical signs observed only in one additional pigeon. Lymphoplasmacytic interstitial nephritis was detected in infected pigeons as well as in non-infected-controls. The relative area of nephritis (aon) in the non-infected controls was between 0.19-1.91 %, compared to 0.01-19.37 % in experimentally infected pigeons and infected sentinels. The sentinel pigeons and the one pigeon suffering prolonged disease had aon-values above 4 %, whereas the pigeon that died acutely, and three clinical healthy pigeons had aon-values below 0.3%.

Discussion (and/or Conclusions): Computer assisted quantitative image analysis was a useful tool to grade interstitial nephritis in pigeons. Histopathological and immunohistochemical data will be discussed with regard to the course of PPMV-1 infection.

Notes:

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2b Fundamental Research	Thu, 1:15 – 2:15	P47	Achim Gruber	P47 – P55
3a Oncology III & Immunology	Thu, 5:00 – 6:00	P56	Andreas Beineke	P56 – P67
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Poster Presentations ESVP/ECVP

P001 INTRAVASCULAR T-CELL LYMPHOMA IN A FJORDHORSE

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Introduction: Intravascular lymphomas are rare tumours characterised by proliferation of lymphoid neoplastic cells only within the lumina of blood vessels. In humans these tumours arise mainly from B-cells. In veterinary literature, only one case of a Thoroughbred with an angiotropic T-cell lymphoma has been described so far.

Materials and Methods: A 23-year-old Fjordhorse gelding with a one-week history of weight loss and inappetence was euthanised after clinical examination, blood work and transabdominal ultrasonography. The horse was submitted for pathologic examination. Immunohistochemistry for B- and T-cells was performed.

Results: Main clinical findings were depression, anorexia and pale mucous membranes. Ultrasound revealed mild peritoneal effusion diagnosed as haemascos with acute bleeding and a paramedian located, irregular, abdominal mass with mixed echogenity. Blood work yielded macrocytic anaemia, thrombocytopenia, leukocytosis without signs of malignancy, hypalbuminaemia and elevated alkaline phosphatase- and lactate dehydrogenase-activities. At necropsy the mass was diagnosed as severe haemorrhage reaching from subcutaneous to subperitoneal tissue with focal rupture of the peritoneum and subsequent haemascos (5 L). Histology revealed no neoplastic tissue within the mass, but numerous CD3-positive and CD79α-negative lymphoblasts within small blood vessels of lung, kidney, bone marrow sinusoids and liver sinuses. No tumour mass was found outside the vascular system.

Discussion: The rare entity of an intravascular T-cell lymphoma was diagnosed in a Fjordhorse. Anaemia was most likely due to extravascular haemolysis, exacerbated by the blood loss into the peritoneal cavity. The cause of the widespread haemorrhage within the ventral abdominal wall may have been a traumatic insult.

Notes:

P002 THREE HORSES WITH MULTICENTRIC T CELL-RICH B CELL LYMPHOMA AND NEUROLYMPHOMATOSIS

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Introduction: Neurolymphomatosis represents a rare manifestation of lymphoma with infiltration of tumour cells into peripheral nerves. In this report neoplastic peripheral nerve changes in three horses with multicentric lymphoma are described.

Materials and Methods: Three horses were necropsied and macroscopical, histological, and immunohistochemical investigations were performed. For characterisation of lymphomas antibodies directed against CD3 (T-cell origin) and CD79a (B-cell origin) were applied. Furthermore, to show degenerative axonal changes, an antibody directed against β-amyloid precursor protein (β-APP) was used.

Results: T-cell rich B-cell lymphomas were detected in all three horses. Surprisingly, in all horses an involvement of peripheral nerves was evident. In addition, Wallerian degeneration and axonopathies, characterised by β-APP accumulation, were found.

Discussion: Neoplastic nerve infiltration represents a rare entity but has to be considered as a differential diagnosis for peripheral neuropathy in equine lymphoma with and without central nervous system involvement. The described neoplastic and degenerative changes of peripheral nerves in horses are similar to described findings in neurolymphomatosis of humans and carnivores.

Notes:

Poster Presentations ESVP/ECVP

P003 A PILOT STUDY OF HORSES THYROID TUMOURS IN LITHUANIA

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Introduction: Tumours of the thyroid gland tend to occur more frequently in warm-blooded and aged horses than in young ones. Most of them are of follicular epithelial origin. The aim of this work was to show frequency and type of thyroid tumours in Lithuanian cold-blooded horses.

Materials and Methods: Samples were obtained from 50 cold-blooded horses selected randomly at the slaughterhouse with no history of endocrine system diseases. There were 54% (n = 27) females and 46% (n = 23) males from 2 to 28 years old. Samples were taken from both sides and fixed in 10% neutral buffered formalin, processed routinely and stained with haematoxylin & eosin and Sirius red.

Results: 14 horses (11 females and 3 males) had one or more different tumours; in total: 23 cases. Three horses with tumours were 2-5 years old; other horses were from 11 to 28 years old. Adenoma was found in 39% (n = 9) cases, adenocarcinoma in 52% (n = 12), fibrosarcoma in 4.5% (n = 1) and granular cell tumour in 4.5% (n = 1) cases. From 12 cases with adenocarcinomas 10 cases were tubular carcinoma, 1 case with tubular-papillary carcinoma and one clear cell type carcinoma.

Discussion and Conclusions: In this study thyroid adenocarcinomas occur more often than adenomas. Females had more thyroid tumours, than the males; older horses more frequently, than younger ones. UNICEF declared Lithuania as an iodine deficiency region. Reports suggested that incidence of thyroid tumours is highest in the areas that are iodine deficient.

Notes:

P004 SOFT TISSUE TUMOUR OF THE FRONTAL SINUS AND BRAIN CAVITY IN A COW

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Introduction: Tumours of the head are not very common in cattle. The aim of this work is to present a rare case of a soft tissue tumour occupying the frontal sinus, growing into the bone and extending into the brain cavity.

Materials and Methods: Neurological clinical symptoms were diagnosed at ante-mortem examination of a 12 year cow. A huge neoplasia was found at necropsy when the head was opened. Tumour was examined with histopathology and immunohistochemistry and tumour grade was determined using grading system for soft tissue sarcoma.

Results: Tumour was encapsulated, growing into or from the right frontal sinus, extending over the midline to the left side, invading bone and bulging into the brain cavity, where it was positioned between dura and bone. Tumour mass formed deep impression into the cortical tissue on the right side of the brain without tissue invasion. The right frontal and temporal bone were thinned and had several pathological fractures. Head muscles, fasciae and lymph nodes of the head were free of tumour. Histopathology showed Grade 2 spindle-cells soft tissue tumour with considerable atypia, nuclear pleomorphism and a small number of mitoses. Majority of tumour cells were positive for vimentin and S-100 protein, some cells expressed a-smooth muscle actin and a few scattered cells were positive for glial fibrillary acidic protein.

Discussion and Conclusions: The origin of the tumour was not clearly established. Localisation, morphology and immunohistochemistry matched mostly to the tumours of peripheral nerves but with some characteristics of meningioma as well.

Notes:

Poster Presentations ESVP/ECVP

P005 A CUTANEOUS MAST CELL TUMOUR IN A LLAMA (LAMA GLAMA)

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Introduction: Mast cell tumours (MCTs) are classified among cutaneous round cell tumors in animals and have been rarely reported in llamas so far.

Materials and Methods: A 3-year-old male-castrated llama had an ulcerated, 4 cm in diameter, cutaneous, soft, well-demarcated and not-movable mass on the lateral aspect of its left lower hind limb. Otherwise the llama was clinically healthy. The mass was surgically removed, fixed in formalin and submitted for histological and immunohistochemical evaluation.

Results: H&E staining revealed an intradermal mass composed of sheets of densely packed, round to polygonal cells. The cells had distinct cell borders, a moderate amount of pale, amphophilic cytoplasma and a round centrally located nucleus, with up to two small nucleoli. Anisokaryosis and anisocytosis were mild. Mitoses were 0 to 1 in 10 high- power fields (x400). Scattered among neoplastic cells there were few eosinophil granulocytes, and clusters of small mature lymphocytes were located at the periphery of the mass. Neoplastic cells had few Giemsa and Toluidine blue-positive intracytoplasmic, metachromatic granules. In immunostained sections, neoplastic cells were diffusely positive for c-KIT and mast cell tryptase, and negative for cytokeratin.

Conclusion: A final diagnosis of mast cell tumour was established, based on histological, histochemical and immunohistochemical results. Due to the well-differentiated morphology, the mild atypia of the neoplastic mast cells and the complete excision, a presumably benign behaviour was formulated. The llama showed no recurrence up to these days.

Notes:

P007 PROMOTER METHYLATION AND PROTEIN EXPRESSION OF DNA REPAIR GENES IN SQUAMOUS CELL CARCINOMA ACCOMPANYING *CANDIDA ALBICANS* INFECTION AND CHRONIC INFLAMMATION IN THE FORESTOMACH OF ALLOXAN-INDUCED DIABETIC RATS

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Introduction: We reported that alloxan-induced diabetic rats have proliferative lesions of the squamous epithelia in the forestomach with chronic inflammation and *Candida albicans* infection, and that some progressed to squamous cell carcinoma (SCC). However, precise mechanism of carcinogenesis remains unclear in this model. In the present study, we try to investigate the promoter methylation and protein expression of DNA repair genes to analyse whether the epigenetic changes may involve in carcinogenesis in this model.

Materials and Methods: Using laser capture microdissection, squamous cells in 1) normal, 2) hyperplasia, 3) hyperplasia adjacent to SCC and 4) SCC were dissected from the forestomach in alloxan–induced diabetic WBN/Kob rats. The four DNA repair genes (BRCA1, ERCC1, XRCC1, MLH1) were analysed by methylation specific PCR, and their protein expressions were examined immunohistochemically.

Results: All genes were not methylated, but unmethylation on BRCA1 and ERCC1 genes were attenuated in the carcinogenic process of SCC. In immunohistochemical analyses, BRCA1 protein was expressed in the nucleus of normal squamous epithelia, and disappeared in squamous hyperplasia and SCC. ERCC1 protein was positive in the nucleus of normal squamous epithelia, and disappeared in squamous hyperplasia. However, nucleus in SCC regained immuno-positivity against ERCC1 protein.

Conclusions: The promoter methylation of 4 DNA repair genes was not detected, whereas the protein expression varied in the carcinogenic process of SCC.

Notes:

Poster Presentations ESVP/ECVP

P008 EFFECT OF THE DOWNREGULATION OF MAST CELL INFILTRATION AND KIT RECEPTOR EXPRESSION IN THE TRANSGENIC PROSTATE CANCER MURINE MODEL (TRAMP)

Notes:

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Introduction: KIT (CD117) is a transmembrane receptor, with tyrosine kinase activity, and its activation is critical for mast cell homeostasis and function. Mast cells (MCs) play a key role in cancer thanks to molecules they secrete that benefit tumour growth and progression which disrupt the surrounding matrix, stimulate angiogenesis and facilitate metastases. MCs increase during the progression from intraepithelial neoplasia to invasive and metastatic prostate cancer in the TRAMP model.

Material and Methods: TRAMP mice were treated with masitinib, a tyrosine kinase inhibitor. Primary antibody reactivity for immunohistochemistry was detected by UltraVisionLP HRP polymer & DAB PLUS kit (Thermo). Real-time PCR analysis for c-Kit mRNA was performed in formalin fixed paraffin embedded samples by using the ARN totalRecoverAll ™ (Ambion, TX, USA).

Results: A significant decrease of MCs number and KIT receptor expression was detected in the prostate of masitinib treated TRAMP mice correlating with a decrease in cell proliferation, tumour angiogenesis, lymph node and lung metastasis. Only apoptosis was increased. The tumour progression was delayed in comparison to controls.

Conclusions: Mast cell infiltration, cell proliferation, angiogenesis and metastasis were decreased in the TRAMP model, by down regulating KIT receptor expression.

P009 HISTOPATHOLOGICAL AND IMMUNOHISTOLOGICAL EVALUATION OF ANAPLASTIC LARGE-CELL LYMPHOMA WITH *EPSTEIN-BARR VIRUS* IN AN ORANGUTAN (PONGO PYGMAEUS)

Notes:

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Introduction: Anaplastic large cell lymphoma (ALCL) originated from T-cell or null-cell lineage, has been included in peripheral T-cell lymphomas by the World Health Organisation (WHO) classification published in 2007. In this study, viral antigen such as *Epstein-Barr virus* (EBV) was detected in an orangutan, though the aetiological association with above viruses and ALCL could not be exactly identified. Also, information on various immunohistochemical markers and serological, virological, clinicopathological, and histopathological data is provided. Additionally this is firstly reported case of ALCL in an orangutan.

Materials and Methods: The animal, which had been raised in a zoo, was submitted for post mortem examination and gross findings were recorded. Histopathological, immunohistochemical, and virological studies were carried out.

Results: Histopathologically, the neoplastic cells were small to large and had round to oval nuclei with hyperchromatic or clumped chromatin. Some tumour cells had horseshoe-shaped or multinucleated nuclei. Half of cells had 1~2 conspicuous basophilic nucleoli. Mitotic figures were frequently observed. Immunohistochemically, the neoplastic cells were uniformly positive for CD30 with staining restricted to the cytoplasm and cellular membrane and strongly positive for CD3. However, CD56 and CD79a were negative in the tumour cells. Herpes virus DNA including *Herpes simplex virus* (HSV) types 1 and 2, *Epstein-Barr virus* (EBV), and *Cytomegalovirus* was detected in submandibular lymph node. Additionally, serological test of HSV, *Human immunodeficiency virus* (HIV), and *Mumps virus* were negative.

Discussion (and/or Conclusions): We report a rare spontaneous case of ALCL with lymphadenopathy and metastasis in an orangutan. Also, EBV antigen was demonstrated in tumour mass though the correlation of ALCL and EBV cannot be proved as the single case of spontaneous lymphoma and the absence of other tumour cases in animals.

Poster Presentations ESVP/ECVP

P010 A CASE OF ADENOCARCINOMATOSIS IN COMMERCIAL LAYER WITH EGG APICAL ABNORMALITY (EAA) SYNDROME

Notes:

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Introduction: Adenocarcinomatosis is a neoplastic disease involving primarily the intestinal and reproductive tract. We report a case of adenocarcinomatosis in laying hens, which were also affected by the egg apical abnormality (EAA) syndrome, called *Mycoplasma synoviae*-associated egg-pole shell defect.

Materials and Methods: White/grey, multinodular mass located in the jejunal mesentery and organ samples were taken from 77-week-old hens. Paraffin-embedded tissue samples were examined with routine H-E and additional staining method. ELISA test (blood sample), PCR and Real-Time PCR (palatine fissure swabs and deformed eggs) were used for detection of *Mycoplasma synoviae* infection.

Results: Histologically, neoplastic cells formed tubuloacinar structures that had features of tubular adenocarcinoma. Mitotic figures were rare, but necrosis was evident. Metastatic adenocarcinoma, necrotic and inflammatory foci, accompanied by congestion and thrombosis were noted in the liver. Based on the results of ELISA (max. antibody titer was 26.896), conventional PCR and Real-Time PCR were confirmed the infection of layer hen with *Mycoplasma synoviae*.

Discussion (and/or Conclusions): Adenocarcinomatosis is generally seen incidentally during necropsy. The aetiopathogenesis of adenocarcinomatosis in layer is still unknown but a relationship between high egg production has been suggested rather than infectious factor. No description of adenocarcinomatosis in EAA-affected layer has been found in the available literature.

P011 THE EXPRESSION OF VIMENTIN AND MINICHROMOSOME MAINTENANCE PROTEIN 3 (MCM3) IN THE TUMOURS OF LEYDIG CELLS OF DOG AND HUMAN – A COMPARATIVE STUDY

Notes:

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Introduction: Testicular tumours in both dogs and humans are the group of more frequently diagnosed cancer lesions within the reproductive system. According to the WHO classification the tumours of Leydig cells in dogs are considered to be one of the most frequently occurring. The aim of the study was to demonstrate the expression of vimentin which is useful in determining the mesenchymal origin of the tumour and MCM3, a supportive protein in the assessment of cell proliferation and to compare the expression of these markers in testicular tumours in dogs and men.

Materials and Methods: Tumours of the dogs (40 samples) and tumours of the men (10 samples) from the archival collections were cut into 4µm-sections. Immunohistochemistry was performed using the antibodies: Vimentin (clone Vim 3B4, DAKO) and MCM3 (clone 101, DAKO).

Results: Vimentin in 85% of Leydig cell tumours gave the strong cytoplasmic reaction at a level of +++ and of ++ in 15%. The expression of MCM3 (nuclear reaction) in humans was observed at a level of + in 90% and at the level of + in 10% samples. In canine tumours MCM3 rated at a level of ++ in 85% and at a level of +++ in 15%.

Discussion (and/or Conclusions): The analysis of the expression level of the tested markers in the testicle tumours in dogs increases the knowledge of the biological behaviour of these proteins in animals and indicates the usefulness of vimentin and MCM3 as diagnostic markers for testicular cancer in both men and animals.

Poster Presentations ESVP/ECVP

P012 THE EXPRESSION OF MINI-CHROMOSOME MAINTENANCE PROTEIN 7 (MCM7) IN A CANINE POORLY DIFFERENTIATED PROSTATE CANCER

Notes:

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Introduction: Neoplastic lesions in prostate are most common in dogs aged about 10. Waters et al. suggest that dogs are the best animal model to examine the factors which affect the initiation and progression of prostate cancer because they live in the same environment as man. According to the World Health Organisation (WHO) prostate cancer in domestic animals is divided into adenocarcinoma and a poorly differentiated cancer. The aim of the study was to determine the expression of MCM7 cell proliferation marker. This protein is present in all phases of cell division and disappears only in the G_0 phase.

Materials and Methods: Sections of cancerous prostate (5 cases) were fixed in 7% formalin, then formed into paraffin blocks and cut into thick fragments of 4µm. Immunohistochemistry was performed using Minichromosome Maintenance Protein 7 (MCM7) (clone DCS-141.1, NOVOCASTRA). The expression of the tested protein was assessed on the basis of the percentage of positively stained nuclei.

Results: The expression of MCM7 in the cells of a canine poorly differentiated prostate cancer was evaluated in all cases at a high level + + + (70-85% of cells showing the positive nuclear reaction).

Discussion (and/or Conclusions): The obtained results indicate a high mitotic potential of this type of cancer and thus considerable aggressiveness. The study also confirmed the usefulness of MCM 7 in the diagnosis of prostate cancer in dogs.

P013 COMPARISON OF CELL PROLIFERATION MARKERS IN CANINE FIBROSARCOMAS

Notes:

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Introduction: Fibrosarcoma is a malignant neoplasms originating from mesenchymal tissue. The most commonly used cell proliferation markers are Ki-67 and PCNA, whose presence can be detected in all cell division phases. PCNA positive reactions can be also disclosed during the DNA-repairing processes and after mitosis is complete. TP53 protein is responsible for cell division and apoptosis regulation. MCM-protein group are initiate DNA duplication.

Materials and Methods: Collected material (20 canine fibrosarcoma tumours) was fixed in formalin and embedded in paraffin blocks, then cut into 4µm-thick paraffin sections, cleared in xylene and passed through alcohols row. Antigens were retrieved in a citrate buffer. Endogenous peroxidase was blocked in 3% hydrogen peroxide solution. The sections were overlayed with primary antibodies: Ki-67 (clone MIB-1, Dako®); PCNA (clone PC10, Dako®); TP53 (clone 318-6-11, Dako®) and MCM-3 (clone 101, Novocastra). Cell markers expression was evaluated using a semi-quantitative scale.

Results: Ki-67 expression in 65% cases was at the level of ++, 25% +++ and 10% +, similar results were obtained in MCM-3 examination: 75% ++, 20% +++ and 5% +. PCNA strength of expression in 85% cases was at level of +++ and 15% ++. TP53 expression in 65% cases was at level of ++, 15% + and lack of reaction in 20%.

Discussion (and/or Conclusions): Conducted research may indicate the usefulness of examined proteins. However, due to PCNA high expression level not only during cell division and lack of TP53 expression in 20% of examined tumours, these proteins results should be regarded with caution.

Poster Presentations ESVP/ECVP

P014 IMMUNOHISTOCHEMICAL DETECTION OF CD25+ LYMPHOCYTES IN FELINE INJECTION-SITE SARCOMA AND POST-INJECTION PANNICULITIS

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Introduction: CD25 is the α-subunit of the membrane-bound interleukin-2 receptor, which is mainly expressed by regulatory T cells (Tregs). Normally these cells regulate immune system activity and prevent autoimmunity. Imbalanced function or number of Tregs, either enhanced or decreased, might lead to tumour development and autoimmunity, respectively. In the present study we hypothesised the presence of CD25+ cells among tumour infiltrating lymphocytes (TILs) that are normally associated with feline injection-site sarcoma (FISS). To this end we have analysed CD25 protein expression by immunohistochemistry in eighteen cases of FISS and in 15 cases of post-injection panniculitis (PIP).

Materials and Methods: 4 µm sections were immunostained by avidin-biotin technique for CD25 (MAb). Staining was evaluated semi-quantitatively for percentage of positivity (10 fields at 40X) and the expression was considered as follows: percentage ‹20% = weak positivity; 21-50% = moderate positivity; ›50% = strong positivity.

Results: All the FISS specimens and 14 of 15 PIP specimens had lymphocytes positive for CD25. The immunolabeling appeared as distinct membrane staining or as diffuse cytoplasmic expression. 9 FISS cases strongly expressed CD25; the medium expression percentage for all the 18 cases was of 55%. 6 PIP had strong positivity, with a medium expression percentage for all the cases of 41%.

Discussion (and/or Conclusions): The present study identified large proportions of CD25+ cells both among TILs and panniculitis. As observed in experimental animal models, CD25+ lymphocytes inhibit Th1 and CD8+ immune response and consequently they could represent a permissive and stimulatory factor for tumour development.

Notes:

P015 COLLISION TUMOUR IN A DOMESTIC SHORT HAIR CAT

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Introduction: Collision tumours are rare and are composed of two or more independent neoplasms. In this case a collision tumour of a fibrosarcoma and a lymphoma in a cat is described.

Materials and Methods: A 12-year-old cat was presented with a tumour at the right thoracic wall, covered by haired skin and measuring 5 cm in diameter. Due to the location, age and species the clinician suspected a fibrosarcoma. The tumour was surgically removed and sent to the Institute for Veterinary-Pathology, Giessen, Germany, for a pathologic examination.

Results: The tumour was composed of two components: a spindle cell component growing in interlacing streams and bundles and a population of neoplastic blastoid round cells. Without any definable demarcation both populations of neoplastic cells infiltrated into each other, giving rise to a collision zone displaying small foci of round cells adjacent to spindeloid neoplastic bundles. Additionally, infiltrating reactive lymphocytes, areas of necrosis and dystrophic calcification were present within this zone. Immunohistology identified the round cell component as T cells, whereas the spindle cell component was positive for vimentin and negative for markers of peripheral nerve sheet tumours and myocytes.

Discussion: Fibrosarcomas as well as malignant lymphomas are common neoplasms in cats. Although the chance of a concurrent occurrence of both tumour types in the same location is very low, the possibility has to be taken into account by the pathologist, particularly with regard to the different biological behavior and prognosis of these two entities.

Notes:

Poster Presentations ESVP/ECVP

P016 THREE DIFFERENT MALIGNANCIES IN A CAT

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Introduction: A ten years old, obese, 9.4 kg, male Norwegian Forest Cat showed anemia and pain in the left stifle area. The cat was anesthetised to obtain diagnostic biopsies. It died the morning after surgery and was submitted for necropsy. The cat was serologically negative to FeLV and FIV.

Macroscopical findings: The cat was obese. The spleen was severely enlarged, 20 cm long and 3-4 cm thick with a firm texture. The liver had a few small light foci. One half of the right kidney was enlarged and had a crumbling texture. About 15 cm of the small intestine had a severely thickened wall, with crumbling texture. The mesenteric lymph node was enlarged. There was a small, irregularly lytic area in the left proximal tibia.

Microscopical findings: Massive infiltration of neoplastic lymphocytes was seen in the kidney, lymph nodes, bone marrow and intestine. The spleen was severely scirrhous and with scattered foci of irregular cysts, which were delineated by malignant squamous epithelial cells that were acantholytic and eventually necrotic. Neoplastic acantholytic squamous epithelium was also found in the proximal tibia, invading the bone. The spleen also had several foci of fatty tissue with myelopoietic activity. In the liver there were multiple foci of malignant bile duct epithelium.

Discussion: The finding of three different malignant tumours in a cat is very rare. The primary squamous cell tumour was not found and the clinical history of this cat is not fully known yet.

Notes:

P017 FELINE COCCYGEAL TERATOMA – IMMUNOHISTOCHEMICAL CHARACTERISATION OF MATURE AND IMMATURE TISSUE COMPONENTS

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Introduction: Congenital teratomas arising in the coccygeal region are not documented in domestic animals. This report describes a congenital coccygeal teratoma in which mature and immature cells and the expression of Sox2, a regulator of stem cell proliferation and differentiation was traced.

Materials and Methods: A 6-month-old male Burmese cat presented a mass expanding ventrally to 5th–7th caudal vertebrae. The mass was present at birth, excised surgically and fixed in 10% formalin. Samples were processed for histopathology and immunohistochemistry. Primary antibodies against vimentin, pan-neurofilament, $p75^{NTR}$, Sox2, GFAP, periaxin and cytokeratins were used.

Results: Histologically, the tumour was composed of derivatives of all three primordial germ layers with neuroectodermal predominance mainly neural crest differentiation. Double immunolabelling identified Sox2-positive cells co-expressing the neural crest stem cell markers vimentin and $p75^{NTR}$. An overlapping expression of vimentin-negative and Sox2-, $p75^{NTR}$-positive cells and GFAP- and $p75^{NTR}$-positive cells indicated a transition phase from immature to mature non-myelinating Schwann cells (SCs). Periaxin-positive myelinating SCs surrounding neurofilament-positive axons were observed. Sox2 was additionally expressed in immature odontogenic epithelium and cells of endodermal origin. Sox2 was observed in mature satellite glial cells and mucous glands.

Conclusions: This case represents a unique teratoma localised in the coccygeal region of a cat. Sox2 was found in immature SCs, immature odontogenic and endodermally-derived epithelium. However, Sox2 alone is not a specific marker of immaturity, as it was detected in mature non-myelinating SCs, mucous glands and satellite glial cells. Thus, co-localisation of Sox2 with other cell markers is necessary for accurate identification.

Notes:

Poster Presentations ESVP/ECVP

P018 A SYSTEMATIC STUDY ON BOVINE HEARTS AFFECTED BY RIGHT ATRIAL ANEURYSMS

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Introduction: Atrial aneurysms are frequently reported in young cattle as small expansion along the free margins of the right appendage. The authors describe the results of a systematic study on atrial aneurysms, providing some hypothesis about the aetiopathogenetic mechanism.

Materials and Methods: From June 2013 to May 2014 a systematic macroscopic and histological study of 86 bovine hearts affected by aneurysms and 13 not affected (controls) was performed. Affected animals belonged to several breeds (Charolais, Blonde d'Aquitaine, Piedmontese), but mainly cross-breed, regularly slaughtered at the Turin slaughterhouse.

Results: Affected animals were beef cattle 10-30 months old (34.9%; 0.048 prevalence) and veal calves 4-9 months old (65.1%; 0.032 prevalence). The observed aneurysms involved the right atrium and particularly the edge, the upper and lower surface. They were round, single isolated or organised in small clusters, sometimes filled with blood, sometimes empty, with a diameter of 2-6 mm. Histologically it was possible to differentiate two types of aneurysms: parietal or originated from the dilatation of the pectinated muscle arterioles. Affected hearts showed different degrees of left ventricular hypertrophy, arteriosclerosis and disseminated Anitschkow-type nuclear configurations in intramural small arteries. Milder degenerative lesions were present in control hearts too.

Discussion (and/or Conclusions): Several mechanisms could be involved in the aetiopathogenetic mechanism underlying the two types of aneurysms. A genetic predisposition or a congenital connective tissue defect, but especially an increased arterial blood pressure for a cardiac "overwork" represent the most important hypothesised mechanisms.

Notes:

P019 BILATERAL GASTROCNEMIUS RUPTURE IN FRIESIAN CATTLE IN SANTA CATARINA STATE, BRAZIL

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Introduction: Recumbency is a common condition in dairy cattle practice. Clinicians must differentiate several pathologies to reach a conclusive diagnosis, such as toxic (ionophores and *Cassia* spp. poisoning) and nutritional myopathies (deficiency of Selenium or Vitamin E), downer syndrome, traumas and others.

Materials and Methods: A recumbent, 5-month-pregnant Holstein-Friesian heifer was presented for diagnosis. The animal was alert, although unable to stand. It was euthanised due to the poor prognosis and a necropsy was conducted.

Results: The animal was unable to maintain both pelvic members extended straight, and couldn't raise the tarsal joint off the ground. Externally, gastrocnemius muscle area showed severe protrusion and mild crepitation. Tail paralysis and urine retention in the bladder were also observed. At the necropsy, severe diffuse haemorrhagic necrosis with oedema and emphysema were observed in both gastrocnemius. In the ventral area of the S2 vertebra, a fracture of two centimeters was present.

Discussion (and/or Conclusions): Rupture of the gastrocnemius muscle can occur due to vigorous contraction, increasing the intramuscular pressure and volume, originating the collapse of the venous outflow, leading to ischaemia and subsequent rupture. We believe pain from the sacral fracture led to difficulty in standing up, requiring an extra effort from the muscles, which caused the spontaneous rupture of both gastrocnemius muscles. The posture adopted by the heifer, although indicative of this rupture, did not match with the loss of the urinary bladder control, making clinical diagnosis difficult.

Notes:

Poster Presentations ESVP/ECVP

P020 HYPERTROPHIC CARDIOMYOPATHY IN SPANISH PUREBREED HORSES

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Introduction: Cardiac disease is considered the third most common cause of "poor performance" in athletic horses (after musculoskeletal disease and respiratory disorders); however, cardiac abnormalities are rare. Horses with cardiac dysfunction typically present with a history of poor performance/exercise intolerance, distended veins, swelling of the limbs, weakness, or collapse. The aim of this study was to describe cardiomyopathy in asymptomatic Spanish Purebred horses.

Materials and Methods: Necropsy was performed on 15 horses (8 males and 7 females), with ages between 4-15year old. Samples of tissue were collected from the heart and were prepared and stained with Haematoxylin & Eosin (H&E).

Results: On necropsy 9/15 cases revealed hypertrophic cardiomyopathy, specifically left ventricular hypertrophy concentric severed, with left ventricular obliteration. The average diameter was approximately ±6.5 cm. Macroscopic lesions were an increased mass of the left ventricle with increased thickness of the wall and a remarkable increase of the papillary muscle. The thickness of the wall of the left ventricle was on average ±5.5 cm. Regarding the right ventricular wall thickness was on average 3 cm, while the left and right atria were ±2.7 cm on average. Microscopically myocytes are enlarged, with increased size of fibers and hypertrophy and disarray of myofibrils as well as an increase in myocardial degeneration and focal fibrosis.

Discussion (and/or Conclusions): In conclusion we report asymptomatic hypertrophic cardiomyopathies in Spanish Purebreed horses, specifically concentric left ventricular hypertrophy.

Notes:

P021 DEFORMATION OF DORSAL CRESTY NECK IN DONKEYS A PATHOLOGICAL STUDY

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Introduction: This report presents a pathological study of deformation dorsal cresty neck in donkeys.

Materials and Methods: Twenty five donkeys (*Equus asinus*) Andalusian Breed (15 male and 10 female), between 8-18 years old, with severe deformation of dorsal cresty neck were studied. A clinical examination was performed and necropsies undertaken with tissue samples processed for conventional histolopathological procedures. .

Results: All cases were obese and had chronic laminitis.Necropsy revealed ventral oedema, lipid accumulation in subcutaneous tissue and xanthomatosis. Muscular steatosis in cervical muscles was observed, involving fat replacement of muscles fibres. Besides muscular steatosis in the dorsal region of the neck, lipomatosis was observed in cresty neck, characterised by diffuse thickening of the subcutis by mature adipose tissue. The overlying epidermis and dermis were mildly atrophic. The subcutaneous fat showed a normal complement of fibrous septa, blood vessel and nerves. The interlobular septa showed mild mucin deposition. The majority of lipocytes were normal and a small number of primitive mesenchymal cells and lipoblasts were present around blood vessel.

Discussion (and/or Conclusions): In conclusion, we describe the pathological features of lipomatosis and muscular steatosis causing deformation of the dorsal cresty neck in donkeys. Multidisciplinary studies are needed in donkeys with this deformity considering aetiology, pathophysiology, incidence and associated factors and their economic impact.

Notes:

Poster Presentations ESVP/ECVP

P022 DORSAL CRESTY NECK IN SPANISH PUREBRED HORSES A PATHOLOGICAL STUDY

Notes:

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Introduction: The deformation of the dorsal edge of the neck (DBDC) is a condition with clinical and economic impact, which presented in Spanish purebred horses and other breeds such as the Lusitano.The aim of this study was to undertake a pathological study of dorsal cresty neck in Spanish Purebred horses.

Materials and Methods: 10 horses (*Equus caballus*), from Cordoba, Spain were studied. All were Spanish Purebred horses (7 male and 3 female), between 5-10 years old. Following clinical evaluation samples were obtained at necropsy and processed by conventional histological procedures.

Results: Necropsy revealed excessive lipid accumulation in subcutaneous tissue in the cresty neck. Microscopically lipomatosis was observed, characterised by diffuse thickening of the subcutis by mature adipose tissue. The overlying epidermis and dermis were mildly atrophic. The subcutaneous fat showed a normal complement of fibrous septa, blood vessel and nerves. The interlobular septa showed mild mucin deposition. The lipocytes were normal and a small number of primitive mesenchymal cells and lipoblasts were present around blood vessels.

Discussion (and/or Conclusions): In conclusion, we describe lipomatosis in the dorsal cresty neck in Spanish Purebred horses.

P023 MAXILLOFACIAL FIBROUS OSTEODYSTROPHY IN A MARE – A CASE REPORT FROM COLOMBIA

Notes:

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Introduction: Fibrous osteodystrophy is a metabolic disorder of calcium in animals and humans characterised by bone resorption and proliferation of conjunctive tissue due to feeding diets high in calcium oxalates or low in calcium and high in phosphorus. The calcium imbalance stimulates parathormone secretion by parathyroid gland chief cells, leading to bone resorption. Here we report the clinical and pathological findings in a mare suffering from the disease.

Materials and Methods: A 14-year-old pregnant mare developed poor body condition (2/5), swollen face and oral lesions while grazing in kikuyo grass (*Pennisetum clandestinum*). The animal presented bulging of nasal and maxillary bones, mouth lacerations associated to spicules in the surface of molars and premolars and easily fragmented teeth; other bones did not show apparent lesions. Analysis of oxalates levels in a sample of kikuyo revealed 3.03 g/Kg of dry forage.

Results: Necropsy examination showed the maxillary and mandibular bones severely softened and the mandible was easily breakable. The parathyroid gland was increased in size. Microscopically the bone tissue was reduced and showed fibrous connective tissue proliferation, multiple irregular and very thin bone spicules, most of them had microfractures and severe osteoclasts-mediated bone resorption. Hyperplasia and hypertrophy of parathyroid chief cells with increased cytoplasm to nucleus ratio and severe vacuolisation were also present.

Discussion (and/or Conclusions): Fibrous osteodystrophy caused by nutritional secondary hyperparathyroidism developed in a pregnant mare by grazing kikuyu grass for long periods of time without appropriate supplements. Kikuyo grass is characterised by high levels of oxalic acid that forms insoluble oxalates responsible for low calcium absorption in the small intestine and the progress of hyperparathyroidism.

Poster Presentations ESVP/ECVP

P024 MORPHOLOGICAL CHARACTERISATION OF MEGAOESOPHAGUS IN FRIESIAN HORSES

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Introduction: Megaoesophagus is a chronic megaoesophagus condition that is quite rare in horses although a predisposition in the Friesian breed has been suggested. For better understanding of the pathogenesis a morphological characterisation of functional megaoesophagus in Friesian horses was performed.

Material and methods: Eighteen Friesian horses clinically diagnosed with megaoesophagus were subjected to necropsy and compared to 3 Friesian and 3 Warmblood control horses. The megaoesophagus was examined for the presence of degeneration and necrosis, inflammation, mineralisation, number of neurons and collagen deposition using histopathology and immunohistochemistry.

Results: Six of the 18 affected Friesians lacked grossly visible megaoesophagus dilatation. Compared with controls, there was significantly more degeneration and necrosis present in both the dilated and non-dilated parts of the megaoesophagus in the clinically affected horses ($P<0.05$). The affected horses had significantly more collagen present between muscle fibers of the muscular layer of the non-dilated megaoesophagus parts than the control group ($P<0.05$). Additionally, these collagen fibers were abnormal, as they were disorganised and presented as small clumped aggregates. The dilated part of the megaoesophagus showed a significant decrease in the number of neurons ($P<0.05$).

Conclusion: Increased amounts of aberrant collagen in the non-dilated parts of the megaoesophagus suggest a connective tissue disorder as a possible underlying cause. Such collagen was also present in the megaoesophagus of neonatal Friesians supporting the hypothesis of a hereditary trait. There may be a relation with Friesians affected with aortic rupture, since aberrant collagen has been implicated in that disease as well.

Notes:

P025 UNDERSTANDING FELINE HEPATIC LIPIDOSIS PHYSIOPATHOLOGY

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Introduction: Feline Hepatic Lipidosis (FHL) represents one of the most common metabolic disorders in cats. Apolipoprotein (apo) A-IV is a protein known to participate in the regulation of various metabolic pathways, with special regards to lipid metabolism. The present study demonstrates a relation between hepatic lipid content and apo A-IV expression in rat and cat liver.

Materials and Methods: Livers from cats (5) and rats (11). Hepatic lipid content was obtained using Mojonnier method. Apo A-IV liver protein expression was assessed by ELISA and confirmed by immunoblotting using liver tissue extracts. Finally, in attempt to graduate also histologically the degree of lipidosis, comparing histological scores with the levels of Apo A-IV expression, samples of all livers used in the study were routinely processed for histopathology and immunohistochemistry.

Results: Even though preliminary, results show an interesting linear relation between hepatic lipid content and apo A-IV liver expression, highlighting differences between cat and rat lipid metabolism.

Discussion (and/or Conclusions): Obtained results, applying this comparative experimental approach, suggest an innovative pathogenetic hypothesis of FHL. In conclusion, the authors propose apo A-IV as a "new" molecule involved in the FHL determinism and its potential role as a prodromal diagnostic biochemical marker.

Notes:

Poster Presentations ESVP/ECVP

P026 MULTIPLE TRAUMATIC NEUROMAS WITH SWOLLEN NERVE FASCICLES AND PERINEURIAL HYPERPLASIA

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Introduction: Traumatic neuroma is a reactive and non-neoplastic proliferative disease, occurs following tail docking in dogs. Microscopically, small nerve fascicles proliferate with abundant fibrous stroma. We encountered multiple traumatic neuroma that was histologically characterised by swollen large-sized nerve fascicles and perineurial hyperplasia at the skin in a dog.

Case: Multiple masses sized about 2 cm diameter at maximum were surgically resected from the left femoral skin in a male Papillon dog aged 11 years. The dog had bitten on the region for two years. At first, small dermal mass was formed and they increased in number. Histologically, the multiple masses were formed around large one from dermis to subcutis. Various-sized swollen peripheral nerve fascicles were observed in each mass. They consisted of thin to unmyelinated nerves with hypertrophic Schwann cells, and the perineurium was thickened. Large mass showed marked perineurial hyperplasia with perineurial fibrosis, including small nerve fascicles. Immunohistochemically, the positive rate of GFAP (Schwann cell marker) and PGP9.5 (axon marker) is approximately equal in almost nerve fascicles. Hyperplastic perineurium was positive for type 4 collagen and NGFR. These lesion showed no significant proliferate activity based on Ki 67.

Discussion: Our case resembled traumatic neuroma except swollen large-sized nerve fascicles and perineurial hyperplasia. The structure of nerve fiber in nerve fascicle was similar to traumatic neuroma. In addition, perineurial hyperplasia and fibrosis were reported in Morton's neuroma which was one subtype of traumatic neuroma. Thus, our case was diagnosed as unique subtype of traumatic neuroma.

Notes:

P027 CONFIRMED CASE OF CONN´S SYNDROME IN A CAT

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Introduction: Conn's Syndrome (primary hyperaldosteronism) is one of the most common causes of hypertension in humans due to hormonal disorders. However, it is thought to be one of the rarest endocrine disorders in cats with only a small number of feline cases reported to date. In this syndrome, hyperaldosteronism results from unilateral or bilateral nodular hyperplasia, adenomas, adenocarcinomas or diffuse, bilateral adrenocortical hyperplasia of the zona glomerulosa.

Materials and Methods: A 14-year old European shorthair cat with clinical hyperaldosteronaemia and hypokalaemia was euthanised with central nervous abnormalities, neuromuscular dysfunction and suspected renal failure.

Results: Necropsy revealed concentric hypertrophy of the left ventricle, interstitial nephritis with marked glomerulosclerosis and multifocal intravascular thrombi within the vessels of the brain stem and the cerebellum. Additionally, severe myocardial and renal interstitial fibrosis was found and similar fibrotic changes were present in the tunica media of various blood vessels. The adrenal glands had multifocal, bilateral, nodular hyperplasia with intralesional, proliferating cells immunohistochemically positive for aldosterone.

Conclusion: The present case indicates that Conn's Syndrome may cause severe clinical and pathological changes in cats which primarily result from severe hypertension as well as regulative hypokalaemia. Cats with hyperaldosteronism commonly die due to renal and/or cardiac failure, thrombosis or musculoskeletal dysfunction so that histopathological examination with particular regard to the adrenal glands may support the aetiological diagnosis of Conn´s Syndrome.

Notes:

Poster Presentations ESVP/ECVP

P028 PRESENCE OF NERVE GROWTH FACTOR (NGF) AND TYROSINE KINASE A RECEPTOR (Trk A) IN DISEASED CAT KIDNEYS

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Introduction: NGF and his high-affinity receptor Trk A are present both in normal and diseased kidneys in vertebrates, playing an important role in development, growth and response to injury. This study reveals for the first time the presence of NGF and Trk A receptor in diseased cat kidneys.

Materials and methods: Kidney samples from 7 cats were fixed in Bouin's fixative for 24 hours and embedded in paraffin. Biochemical profile and urinary analysis were available for each cat confirming renal disease. The histopathological lesions, were confirmed using HE and periodic acid Schiff stains. Immunohistochemical ABC Elite detection kit (Vector, Burlingame, CA) was used. Finally, the sections have been examined and photographed with a Olympus CX41 microscope.

Results: Histological stains confirmed renal lesions consisting of glomerulosclerosis, mesangial expansion, tubular atrophy and interstitial fibrosis. NGF and Trk A immunoreactivity was observed in renal tubular cells, proximal and distal tubes being especially targeted.

Discussions and/or conclusions: The results confirmed the presence of NGF and Trk A in diseased cat kidneys, demonstrating their possible role during the pathogenesis of tubular injury.

Notes:

P029 COMPARISON OF HISTOPATHOLOGICAL CHANGES IN ATRIAL AND VENTRICULAR SPECIMENS FROM DOGS WITH DILATED CARDIOMYOPATHY (DCM)

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Introduction: Dilated cardiomyopathy (DCM) is a disease of both animals and humans involving myocardial changes causing heart failure. The alterations are present mostly in the left ventricular wall but were also stated in the right ventricular wall and interventricular septum.

Materials and Methods: The study was carried out on a group of 6 dogs (4 doberman pinchers and 2 boxer dogs) with clinically stated dilated cardiomyopathy. Heart specimens from both atria, both ventricles and interventricular septum were stained with HE and Masson-Goldner trichrome. The assessment of presence and intensity of fibrous tissue, adipose tissue, inflammatory infiltrates, vascular changes and cardiomyocytes degenerative changes was made.

Results: Complex histological changes involving both atrial and ventricular walls were noted. Higher intensity of fibrous tissue presence in atria compared to ventricles and no other significant differences were stated.

Discussion: Previous work has indicated that histopathological changes can be found not only in the left but also in the right ventricle. We have compared specimens from all heart chambers showing that alterations affect also the atrial myocardium. The observed higher intensity of fibrous tissue in atria compared to ventricles can reflect high susceptibility of dogs with DCM to supraventricular rhythm disturbances. At this point it was impossible to specify whether the stated alterations are an effect of myocardial cells primary remodelling trend or heart response to progressing overload and heart failure.

Notes:

Poster Presentations ESVP/ECVP

P030 LESIONS INDUCED BY MOTOR VEHICLE ACCIDENTS IN DOGS AND CATS

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Introduction: Motor vehicle-related trauma is a significant cause of death in dogs and cats including tissue disruptions, organ dysfunctions and cellular damages. The present study describes the most frequent gross lesions induced by car accidents in domestic carnivores submitted for examination between 2012-2014.

Materials and Methods: The research included 13 dogs and 9 cats. The animals were submitted to radiologic investigation and necropsy. Supplementary information was provided by the owners, concerning the place and circumstances of the accident, and subsequent therapy prior death.

Results: Majority of dogs was medium-sized breeds and cats were adult. The overall male/female ratio was 1.16/1 for dogs and 1.25/1 for cats. The most injured regions in dogs were the thorax (69%) followed by abdomen (53%). 33% of cases exhibited both thoracic and abdominal trauma. Rib fractures (55%) were frequently associated with pulmonary rupture (33%) and pneumohaemothorax (22%). The abdominal cavity included liver transdiaphragmatic herniation (57%), hepatic and renal rupture (28% and 14%, respectively). Trauma of limbs (23%), spinal cord and pelvis (15%, respectively) were also described. The cats revealed the same dominant incidence of abdominal and thoracic trauma, as described in dogs (55%). Transdiaphragmatic herniation of liver recorded a bigger percentage than dogs (60%). The head and limbs were less involved (11%).

Discussion (and/or Conclusions): The injuries caused by motor vehicle accidents frequently involve the thorax and/or abdomen. Despite the serious injuries of cavities and organs, lack of gross lesions of the skin was noticed in both species. Fragmentation of solid organs of abdominal cavity was associated with impact with high speed driving vehicles.

Notes:

P031 FIBROSIS AND CYSTS IN THE LIVER AND CYSTIC MUCINOUS HYPERPLASIA OF THE GALLBLADDER IN A FERRET – CASE REPORT

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Introduction: Cystic mucinous hyperplasia of the gallbladder is incidental in dogs. We found this lesion associated with fibrosis and cysts in the liver in a ferret.

Materials and Methods: A 2-year-old male ferret showed weakness and anorexia. Biochemical blood analysis revealed significant increase of liver marker enzymes parameters (GOT, GPT). Enlargement of the liver was seen in USG. Animal was continuously treated for hepatic failure and after about 5 years was euthanised. During autopsy specimens of chosen organs were collected and stained with HE, PAS, Massone trichrome.

Results: Necropsy showed a marked enlargement of the right medial lobe of the liver, in which numerous thin-walled cysts of various sizes were present, filled with mucus-like, cloudy liquid, coloured from milk to milk-greenish. In the light microscope cysts were lined by single layer of flat or cuboidal epithelium and filled with amorphous material and dead cells. Significant fibrosis in the remaining small fragment of parenchyma was seen and hepatocytes between the connective tissue showed variable stages of degeneration and necrosis. Vacuolar and fatty degeneration was seen in other lobes of the liver. The gallbladder displayed hyperplasia of mucus-secreting mucosal gland with mucus-filled cysts formation. Cholestasis in the liver, kidneys and pancreas was found.

Discussion: The case presentation and microscopic findings suggest congenital biliary cysts and hyperplasia of mucoid glands in the gallbladder, which have not been described in ferrets. There is possibility of neoplastic transformation of congenital biliary cysts – in another ferret from the same litter adenocarcinoma, originating from bile ducts, was found.

Notes:

Poster Presentations ESVP/ECVP

P032 FRACTAL CHARACTERISTICS OF ADIPOCYTES IN MICE FED WITH HYPERCALORIC DIET

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Introduction: Changes in the morphology of the adipocytes reflect their modified functional status, related with the metabolic state of the individual. The current study investigates if fractal analysis is useful in highlighting the impact on adipocytes of progressive exposure to a hypercaloric diet.

Materials and Methods: A total of 18 NMRI mice were assigned to 3 groups: control group (C), obesity induced by hypercaloric diet at one (M1) and two months (M2). Samples from mesenteric, omental, perirenal and inguinal subcutaneous adipose tissue were collected from each subject. 360 photomicrographs 400x of HE-stained paraffin sections, were digitally processed and their fractal dimension (FD) was calculated. The differences among the averages (medians) of each group – anatomic site were calculated and, when found significant ($P<0.02$), the performance of a differentiation-test based on FD was evaluated by ROC (Receptor Operating Curve) plot analysis.

Results: FD was clearly different (area under ROC curve >0.9) between M1 and C, as well as between M2 and C, regardless the anatomic site examined. Only the inguinal subcutaneous site provided a sound statistical distinction between M2 and M1 (area under ROC curve 0.714), within performance range of current medical tests.

Discussions and Conclusions: FD is an effective, reliable tool for identifying subtle and diffuse changes in the adipose tissue. Although previous reports had hinted at the omental region, the inguinal subcutaneous site was found to provide the most sensitive samples to both emergence and dynamics of changes in the adipose tissue status induced by exposure to hypercaloric diet.

Notes:

P033 HYPERCALCAEMIC NEPHROPATHY WITH SEVERE CALCIFICATION OF THE AORTIC VALVE IN AN L'HOEST MONKEY

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Introduction: Hypercalcaemia causes severe electrolyte disturbances with calcium deposition in internal organs, commonly kidney, heart and lung, potentially leading to organ dysfunction. Excessive intake of Vitamin D or poisoning by rodenticides containing vitamin D increases the uptake of calcium by the intestines which can result in hypercalcaemia. Other causes of hypercalcaemia include primary hyperparathyroidism, hypoadrenocorticism, chronic renal failure with secondary hyperparathyroidism and tumours.

Materials and Methods: An eight and a half year old female l'hoest monkey (*Cercopithecus lhoesti*) from a Swedish zoo died during sedation and was necropsied at SVA.

Results: The main findings at necropsy were multiple firm white plaques in the aortic valve and moderate dilation of the left ventricle. Disseminated petechial bleedings were seen in the lungs. The kidneys were firm with a pale discolouration. Histological examination revealed severe calcification of the aortic valve with fibrosis and granulomatous foreign-body reaction. Furthermore, calcification was detected in the wall of the myocardial vessels along with moderate muscle degeneration and fibrosis. In the kidneys severe tubular calcification and degeneration were observed together with interstitial changes dominated by fibrosis and multifocal chronic inflammation. Calcification was also noted in the vessels of the lungs and spleen.

Conclusion: The monkey was diagnosed with hypercalcaemic nephropathy with degenerative lesions in the heart and severe calcification of the aortic valve. Since no evidence of primary hyperparathyroidism, hypoadrenocorticism or tumours were detected at necropsy, excessive vitamin D intake or rodenticide poisoning was suspected as the cause of the hypercalcaemia.

Notes:

Poster Presentations ESVP/ECVP

P034 PERIVASCULAR PROGENITOR CELLS – POTENTIAL CARDIOMYOCYTE PROGENITORS OR CONTRIBUTORS TO CARDIAC FIBROSIS?

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Introduction: Cardiovascular disease is the number one cause of death globally killing over 17 million people a year of which approximately 40% die from coronary heart disease. Limiting cardiac fibrosis and promoting myocardial regeneration are key therapeutic goals. This study looks at the fate of perivascular progenitor cells transplanted into ischaemically injured hearts with relation to their contribution to regeneration and fibrosis.

Materials and Methods: Both CD146+ CD34- pericytes and CD146- CD34+ adventitial cells were isolated by FACS from human foetal heart. Following purification and expansion in culture cells were examined for expression of perivascular, mesenchymal stem cell (MSC) and cardiomyocyte associated genes and proteins. GFP lentivirally transfected cells were injected into ventricular infarcts induced in NOD.SCID mice (n=10). After 21 days mice were subjected to cardiac ultrasound and hearts were fixed and sectioned for scar assessment and cell tracking.

Results: Both populations express a similar panel of genes and surface proteins consistent with a perivascular lineage (CD146, NG2 and PDFRG-β), a MSC phenotype (CD44, CD73, CD90 and CD105) and an immature cardiomyocyte phenotype (Mef2c & GATA4). The mice receiving adventitial cell transplants demonstrated reduced cardiac performance. Adventitial cells were more readily retained and a majority (64% adventitial & 50% pericyte) in both groups adopted a fibroblast morphology with a minority (3% and 13%) adopting a cardiomyocyte morphology post injection.

Discussion (and/or Conclusions): These findings suggest that the human cardiac perivascular niche harbours progenitor cells with both fibroblast and cardiomyocytes commitment.Whether this represents a single population with polarised differentiation potential or a dual population each with distinct lineage commitment remains to be elucidated.

Notes:

P035 THE VALUE OF CYTOLOGIC EXAMINATION OF CANINE MAMMARY TUMOURS

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Introduction: Canine mammary tumours (CMTs) include a great variety of histopathological differences and therefore the diagnosis of CMTs can be challenging, especially within the cytology smears. The aim of study was to determine the accuracy of diagnostic cytology with histopathology of CMTs, correlation with clinical findings and postoperative outcome.

Materials and Methods: A total of 27 cytological samples were obtained by fine needle aspirate (FNA) or tissue imprints. Histopathologic examination (HP) was performed by HE stain, cytopathological examination (CP) – by Giemsa stain. Histological and cytological grading was evaluated.

Results: CP-HP accuracy for malignant and benign CMTs was obtained in 23/27 cases (85.2%). The sensitivity and specificity was 83.3%, 100% respectively. A proper CP-HP correlation for tumour types occurred in 19/27 (70.4%) samples. The cytological grading and typing correlated with histological assessment, respectively. Diagnosis on cytologic examination was not associated with clinical findings, overall survival (OS), but with a cause of death due to CMTs. Additionally, malignant, simple type of carcinomas (in cytology) tended to decrease OS. Shorter OS was significantly associated with tumour metastases and cause of death due to CMT.

Discussion (and/or Conclusions): We conclude that cytopathological examination is a valuable diagnostic tool in differentiating between benign and malignant mammary lesions and to obtain a pre-operative diagnosis. Cytologic features of CMTs may predict histologic grade. However, CP may not provide valuable prognostic information concerning overall survival in CMT patients.

Notes:

Poster Presentations ESVP/ECVP

P036 THE PRESENCE OF SHORT FORM OF RON/STK TRANSCRIPT COULD BE A PREDICTOR OF POOR OUTCOME IN FELINE MAMMARY CARCINOMAS

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Introduction: RON/stk tyrosine kinase receptor, identified in cat as feline-stk, is activated by MSP and over-expressed in human breast cancer. Human RON gene is able to generate respectively the full length (fl) and the short forms (sf) of the transcripts. sf-RON is generated from an alternative transcriptional start from a second promoter within the intron 10, which preserves the kinase activity of the receptor. The aim of this research was to investigate the expression of both RON and MST and to identify by the presence of the sf-RON transcript in feline mammary carcinomas (FMCs) in relation to clinical-pathological findings.

Materials and Methods: IHC expression of RON and MSP was evaluated on 50 FMCs. To detect sf-RON, RNA was extracted from 47 FMCs and RT-PCR, with primers annealing on exon 10 and exon 11, was performed on cDNA.

Results: IHC expression of RON and MSP was observed in the 68% and 58% of FMCs respectively while the 52% of the cases co-expressed both proteins. IHC expression of RON, MSP or both in FMC was not correlated with clinical outcome. The feline sf-RON was detected in 27/47 FMCs and resulted statistically associated with the poorly differentiated histological grade. Queens with FMC showing the sf-RON had a shorter disease free period and a shorter survival if compared with patients negative for sf-RON

Conclusions: This is the first record of the sf-RON in cats and the presence of this isoform represents a potential poor prognostic indicator in feline mammary carcinomas.

Notes:

P037 PROGNOSTIC IMPACT OF NEOADJUVANT AGLEPRISTONE ON CLINICOPATHOLOGICAL PARAMETERS OF PROGESTERONE RECEPTOR POSITIVE CANINE MAMMARY CARCINOMAS

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Introduction: Neoadjuvant treatment of canine mammary carcinomas with the progesterone receptor (PR) antagonist aglepristone (RU534) has a PR expression-related inhibiting effect on proliferation index (PI). This work analyses the effects of neoadjuvant RU534 in the disease free period (DFP) and overall survival (OS) of female dogs with PR+ mammary carcinoma.

Materials and Methods: Thirty-seven non-spayed bitches with mammary carcinomas were treated with RU534 (n=24) (two doses of 20 mg/kg) or placebo (n=13) before surgery (day 15). PR expression and PI were analysed using PR10A9 and MIB-1 monoclonal antibodies, respectively, in tumour tissue samples taken at day 1. Tumour size, histological subtype and histological grade were assessed. DFP (recurrence free, metastasis free or combined) and OS data were retrieved (24 months). The Kaplan Meier method (GraphPad Prim v5.01) was used.

Results: A significant increase in DFP was observed in PR+ experimental carcinomas of complex and mixed ($n = 13$, $p = 0.02$) histological subtype, with lower tumour size (< 3 cm, $n = 9$, $p = 0.002$), histologic grade I ($n = 7$, $p = 0.03$) and grade II ($n = 5$, $p = 0.02$) and with PI < 10% ($n = 12$, $p= 0.05$). No impact on DFP was observed in PR- carcinomas. Neoadjuvant RU534 had no impact on OS of mammary carcinomas irrespective of PR status.

Discussion (and/or Conclusions): Neoadjuvant treatment of PR+ mammary carcinomas with RU534 increased DFP in a subset of tumours with favourable prognostic indicators but no answer was found in PR- tumours.

Notes:

Poster Presentations ESVP/ECVP

P038 FRACTAL ANALYSIS OF CHROMATIN USED FOR IDENTIFYING BENIGN AND MALIGN LESIONS IN MAMMARY GLAND TISSUE

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Introduction: Semi-automatic taking and processing of histopathological pictures is spreading, but reliable diagnostic tools to match the procedure are yet to be confirmed. One of the most promising instruments for gauging pathological changes in the complex morphology of tissue is fractal analysis, already confirmed in human medicine.

Materials and Methods: Mammary tissue samples were collected from 62 dogs and cats. 1237 histology pictures were labeled for the presence/absence of features characteristic for carcinoma and adenoma in dogs, and, supplementary, for fibroadenomatous change in cats. A statistical evaluation was made for the relation of the histopathology labels with the fractal dimension of the chromatin regions (FDCR) extracted by picture segmentation. Tests based on FDCR were assessed by ROC (Receiver Operating Curve) analysis. Likelihood ratios for the presence of lesion were calculated.

Results: FDCR is a good discriminator for the presence/absence of tumour lesions in the mammary gland. The performance of a test based on FDCR to identify the presence of carcinoma in a histology picture is very good for dogs and excellent for cats. The potential benefits of using distinct structural/textural FDCRs were not confirmed.

Conclusions: Fractal analysis is a reliable tool for the identification of malign and benign lesions in histology pictures of mammary tissue in dogs and cats. The test based on the fractal dimension of the chromatin regions can be integrated in complex diagnostic procedures, either by means of reference ranges, or by Bayesian inference, using likelihood ratios for the presence of a specific lesion.

Notes:

P039 IMMUNOHISTOCHEMICAL STUDY OF CANINE MALIGNANT MAMMARY TUMOURS

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Introduction: Matrix metalloproteinases (MMPs) are a family of proteinases which contribute to a variety of biologic processes including ECM degradation. Owing to their matrix-degrading abilities they were taken in to consideration in tumour invasion and metastasis. The main objective of this research is to study relationship between MMP-9 expression in neoplastic cells and tumour adjacent stromal cells of canine malignant mammary tumours and to investigate capability of this protease as a prognostic tumour marker.

Materials & Methods: 32 canine malignant mammary gland tumours were obtained during surgical treatment and necropsy. Tumours were evaluated for histologic classification according to Goldsmith et al. 2011 method and Elston-Ellis method of histologic grading. Besides, MMP-9 expression in neoplastic cells and adjacent stromal cells was determined using immunohistochemistry.

Results: Expression of MMP-9 in tumours with high histologic grade was higher. In addition, stromal expression of MMP-9 in proliferative, high histologic grade, invasive and metastatic tumours was higher than neoplastic expression. Also, the study revealed that there's a significant relationship between histologic grading of tumour and distribution and intensity of MMP-9 expression. Thus, all grade II and III carcinomas and poorly differentiated carcinosarcomas showed high distribution and intensity of MMP-9 expression in neoplastic and tumour adjacent stromal cells.

Discussion: Level of stromal MMP-9 expression was related to poor prognosis. These results suggested that stromal cells production of MMP-9 contributed to more invasive malignant mammary tumour of female dogs. Furtheremore, evaluation of MMP-9 expression could be a useful prognostic factor.

Notes:

Poster Presentations ESVP/ECVP

P040 COMPARISION OF ANGIOGENIC CELL MARKERS IN GRADE III MAST CELL TUMOUR AND HISTIOCYTOMA

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Introduction: Histological pattern of grade mast cell tumour III (MCT) is similar to histiocytoma, due to frequent lack of cytoplasmatic granules. VEGF (Vascular Endothelial Growth Factor) is considered to be the most specific endothelial cell growth factor, it regulates angiogenesis and stimulates endothelial proliferation. Von Willebrand factor (FVIII) expression is mainly found in healthy and neoplastic endothelial cells; it is found in lymphatic vessels.

Materials and Methods: The study was conducted on 10 samples of canine grade III MCT and 10 samples of histiocytoma. Material was fixed in formalin and embedded in paraffin blocks. Studies were performed in 4µm-thick paraffin sections, cleared in xylene and passed through a row of alcohols of a decreasing concentration to water. Antigens were retrieved in a citrate buffer. Endogenous peroxidase was blocked in 3% solution of hydrogen peroxide. The sections were overlayed with primary antibodies: VEGF (VG1, Dako®) and von Willebrand factor (Dako®). Expression of markers was evaluated on the basis of average vessels number per 5 visual field (hot spots) using magnification of 200x.

Results: During the examination of FVIII and VEGF in grade III MCT, an average of 5 blood vessels was detected per visual field. In histiocytoma cases, VEGF stained an average of 2 blood vessels per visual field and no expression of FVIII was observed.

Discussion (and/or Conclusions): The study revealed positive reaction of FVIII in MCT and lack of it in histiocytoma. It might be concluded that FVIII is a useful cell marker to differentiate round-cell tumours.

Notes:

P041 COX-2 AND PTGES EXPRESSION IN CANINE GRADE III MAST CELL TUMOURS

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Introduction: Mast cell tumours (MCT) are one of the most frequently diagnosed skin tumours in dogs. Important enzymes for prostaglandin synthesis are cyclooxygenase-2 (COX-2) and prostaglandin E synthase (PTGES). Furthermore COX-2 inhibits cellular apoptosis, promotes neoangiogenesis, increases tumour cell motility and invasiveness. PTGES expression is observed in inflammatory reactions, as well as in neoplastic lesions. It is induced by interleukin 1 (IL-1β) and TP53 protein.

Materials and Methods: The studies were conducted on 10 canine neoplastic skin tumours, previously diagnosed as grade III MCT. Material was fixed in 7% buffered formalin and embedded in paraffin blocks. Studies were performed in 4µm-thick paraffin sections, cleared in xylene and passed through a row of alcohols of a decreasing concentration to water. Antigens were retrieved in a citrate buffer. Endogenous peroxidase was blocked in 3% solution of hydrogen peroxide. The sections were overlayed with primary antibodies: COX-2 (clone RBT-COX2, BioSB) and PTGES (Catalog No.: AP07074PU-N, Acris). Cell markers expression was evaluated according to the Remmele scale.

Results: In analysis of expression COX-2 in 100% of tumours a very strong cytoplasmatic reaction at the level of +++, 9 in Remmele scale was detected. Upon evaluation of PTGES expression in all tested tumours a pronounced cytoplasmic reaction +++, 9 in Remmele scale was apparent.

Discussion (and/or Conclusions): Both examined cell markers indicated high expression level in grade III canine mast cell tumour. Continuation of these studies will indicate if there is a connection between malignancy grade and intensity of protein expression.

Notes:

Poster Presentations ESVP/ECVP

P042 CD25 IMMUNOHISTOCHEMICAL EXPRESSION IN LYMPH-NODES FROM DOGS AFFECTED BY MAST CELL TUMOURS PRELIMINARY INVESTIGATION

Notes:

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Introduction: CD25 (alpha-chain-IL2-receptor) has been demonstrated to be selectively expressed in neoplastic and reactive mast cells (MCs) in humans and dogs. Aim of this study is to investigate the immunohistochemical expression of CD25 in MCs in lymph nodes from dogs affected by mast cell tumours (MCTs), to elucidate its possible role in distinguishing neoplastic MCs (i.e. metastasis) from resident MCs.

Materials and Methods: 14 canine cutaneous MCTs and 18 loco-regional lymph nodes were surgically obtained and submitted for histology. Serial histological sections were stained with HE, Giemsa and anti-CD25 monoclonal antibody (ABC method).

Results: All MCTs were CD25 immunolabelled, the overall number of neoplastic cells stained varied greatly and the intensity of single-cell staining varied also within the same tumour. In 14/18 lymph nodes, Giemsa + MCs were detected. CD25 stained MCs in 12/18 lymph nodes. In 4 cases both CD25 and Giemsa staining were negative, in 2 cases single scattered Giemsa + MCs were CD25 negative.

Discussion: The identification of single-cell metastasis in lymph nodes from dogs with MCTs is challenging for pathologists, still it represents crucial indication for clinicians. Giemsa staining is a powerful marker of MCs but it allows no distinction between neoplastic and non-neoplastic MCs. CD25 is a promising marker for activated MCs. In the present study Giemsa and CD25 staining on MCs in lymph nodes were consistent except for two cases, in which single Giemsa+ MCs were CD25 negative. Further studies will be necessary to ascertain if CD25 is a reliable marker of activated/neoplastic MCs in lymph nodes of dogs with MCTs.

P043 HISTOPATHOLOGICAL, IMMUNOHISTOCHEMICAL AND FRACTAL DIMENSION ANALYSIS OF DIFFUSE LARGE B-CELL LYMPHOMAS IN DOGS

Notes:

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Introduction: Malignant lymphomas are common neoplasm of dogs and among them diffuse large B-cell lymphoma (DLBCL) represent ≈20%. The aim of our study was to evaluate and compare results of histopathological, immunohistochemical and fractal dimension (FD) analyses of canine DLBCL.

Materials and Methods: Samples of 45 canine lymphomas were evaluated histologically applying the World Health Organisation (WHO) system of classification of canine lymphomas. All samples were examined immunohistochemically using Pax5 and CD3 monoclonal antibodies. Immunohistochemical reactions were evaluated by light microscope at 40× and 100× magnification to detect the percentage of positive tumour cells. Slides from the tumours classified as DLBCL were submitted to computer-based FD image analysis using 3 different 200x power fields for both Pax5 and CD3 IHC staining.

Results: Among 45 analysed lymphomas, DLBCL comprised 29% (13). In all samples tumour cells predominantly showed positive immunostaining for Pax5 (91%) and minority of cells expressed CD3 (9%). According to WHO, all DLBCL were classified into two morphologic variants, centroblastic (CB) with incidence of 77% and immunoblastic (IB) with incidence of 23%. Mean values of determined FD were for Pax5 immunostaining 1.73 and for CD3 1.36. There were no differences in determined FD values between CB-DLBCL and IB-DLBCL.

Discussion: Our results confirm data that DLBCL presents a considerable percentage of canine lymphomas. Results of histomorphological analysis which showed predominance of CB-DLBCL correspond to literature findings. The differences in determined values of FD indicate that this method may be useful for evaluation of Pax5 and CD3 immunoreactivity in canine lymphomas.

Poster Presentations ESVP/ECVP

P044 EXPRESSION OF THE c-KIT RECEPTOR IN CANINE CUTANEOUS EPITHELIOTROPIC LYMPHOMA AND PRELIMINARY RESULTS OF TOCERANIB TREATMENT IN ONE DOG

Notes:

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Introduction: The tyrosine kinase receptor (c-kit) plays an important role in proliferation, survival and differentiation of haematopoietic progenitor cells. Toceranib phosphate (Palladia) is a c-kit inhibitor approved for the treatment of mast cell tumours in dogs. Toceranib phosphate has also been reported as clinically effective in other neoplasias but has never been used in canine cutaneous epitheliotropic lymphoma (CCL). This study determines the expression of c-kit in 5 cases of CCL and their administration leading to partial remission in a dog with c-kit positive CCL.

Materials and Methods: Serial sections were used in the immunohistochemical analysis of anti-CD3 and anti-CD117 (c-kit) (DAKO) expression. Immunoreactive cells were counted using Image Pro-plus 6.0 software. Six photomicrographs (0.2 mm^2 each) per animal were randomly selected for cell counting. Results were expressed as mean ± SD per animal and as percentage of C-kit+ cells and CD3+ cells.

Results: All CCL were strongly CD3+ (80 to 95% of tumour cells showed a membranous pattern) but c-kit expression was variable (2 to 33% positive tumour cells presented a cytoplasmic or membranous immunoreaction). The dog with the highest expression of c-kit is now on its 5th month of toceranib treatment (2.5 mg/kg q48h), achieving complete and partial remission of oral cutaneous lesions respectively.

Conclusions: Toceranib phosphate and other receptor kinase inhibitors may represent a type of targeted-chemotherapy useful in the treatment of CCL.

P045 EVALUATION OF THE PREDICTIVE VALUE OF TOPOISOMERASE-IIα IN THE SELECTION OF THE OPTIMAL THERAPEUTIC REGIME IN CANINE LYMPHOMAS

Notes:

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Introduction: Topoisomerase IIα (TOP-2A) belongs to a group of enzymes participating in the DNA metabolism. TOP-2A is also a molecular target for cytostatic drugs. The determination of the level of TOP-2A expression may constitute a significant predictive factor facilitating individual selection of chemotherapy for dogs with malignant lymphomas.

Materials and Methods: The aim of this study was the determination of the level of TOP-2A expression in dogs with non-Hodgkin lymphoma in the context of the use of anthracyclines in the multidrug therapy. The material was comprised of sections of the lymph nodes of dogs with non-Hodgkin lymphoma. TOP-2A expression was determined using the immunohistochemical method. The assessment was performed using a computer image analysis system through the determination of the percentage of neoplastic cells displaying a positive reaction.

Results: TOP-2A expression was demonstrated in all the tested lymph nodes. However, significant differences in the number of stained cell nuclei and the intensity of reaction were observed. Assuming that the tumours with higher TOP-2A expression are characterised by higher sensitivity to anthracyclines, the use of this drugs in tumours with low TOP-2A expression seems unjustified.

Discussion (and/or Conclusions): The obtained results indicate that the immunohistochemical assessment of TOP-2A expression can serve as a predictive factor for the treatment with anthracyclines in canine lymphomas and indicate a need to perform further clinical studies.

Poster Presentations ESVP/ECVP

P047 SOX9 AND CK15 STEM CELL MARKERS IN CANINE CUTANEOUS SEBACEOUS LESIONS

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Introduction: Sebaceous glands are specialised cutaneous adnexal glands, which work under constant hormonal control to produce sebum. They represent a source of adult stem cells, that are located in the reserve cell layer. They can give rise to several proliferative lesions and neoplasms (adenoma, epithelioma, and carcinoma). Ck15 and Sox9 represent two stem cell markers, expressed by the adult stem cells of human sebaceous glands, and are expressed in several human sebaceous tumours.

Materials and Methods: Aim of the present study was to investigate the immunohistochemical patterns and levels of expression of Ck15 and Sox9 in a set of canine cutaneous sebaceous lesions.

Results: Few scattered Ck15-positive cells, but no Sox9 immunolabelling were observed in the reserve cell layer of normal glands. Sox9 were selectively expressed in the nuclei of the innermost cell layer of the outer root sheath of canine normal hair follicles. The spectrum of canine sebaceous tumours, from benign to malignant, contained a subpopulation of cells expressing Ck15, while Sox9 was expressed only in malignant sebaceous tumours.

Discussion (and/or Conclusions): Although further studies are needed, it could be suggested that Ck15+ and Sox9+ cells, having some features of stem cells, may play a role in sebaceous tumour development and progression.

Notes:

P048 FELINE T CELL RECEPTOR SIGNALING PATHWAY PROTEINS IDENTIFIED BY LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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Introduction: The T cell receptor signaling pathway in humans is well described, but in cats only a model of the feline signaling cascade deduced from public sequence database data exists. This model postulates a strong homology of the feline cascade to the human counterpart. Nevertheless many of the involved proteins are only predicted from the nucleotide sequence analysis as received from the feline genome project. In this study members of the T cell receptor signaling pathway were identified by mass spectrometry in samples of a feline large granular lymphoma cell line.

Materials and Methods: Tryptic peptides of homogenates of a feline large granular lymphoma cell line were analysed by liquid chromatography-electrospray ionisation tandem mass spectrometry (LC-ESI-MS/MS). Resulting data were searched against the UniProtKB (Universal Protein Resource) database filtered with *felis catus* taxonomy.

Results: Seventeen proteins associated with the T cell receptor pathway were indentified in the results. 15 of these were represented by 2 or more peptides and therefore identification was regarded as valid.

Discussion (and/or Conclusions): Of the 15 identified proteins 2 (CD3 epsilon chain and CD8 alpha chain) are already widely recognised by immunhistochemistry while for the others reports of expression on protein level are lacking. With this study the existence on protein level is confirmed. Two proteins were only represented by one peptide and therefore their expression cannot be regarded as proven by this study, however it remains likely.

Notes:

Poster Presentations ESVP/ECVP

P049 PHENOTYPICAL IN SITU AND IN VITRO CHARACTERISATION OF CANINE DORSAL ROOT GANGLIA NEURONS AND SATELLITE GLIAL CELLS REVEAL THE PRESENCE OF A UNIQUE GLIAL PRECURSOR CELL POPULATION

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Introduction: Dorsal root ganglia (DRGs) neurons have been used as an *in vitro* model in neuroscience in pain, virus, and prion protein research. Furthermore, potential stem cell properties have been demonstrated in DRG satellite cells. Recent studies discussed that dog glial cells are a better model for human glia compared to rodents emphasising the usefulness of dogs as a translational animal model. However, detailed knowledge concerning the structure of canine DRGs and *in vivo* and *in vitro* expression of different structural and functional proteins by resident cells is lacking.

Materials and Methods: Light and transmission electron microscopy, immunohistochemistry and immunofluorescence were used to characterise canine DRG neurons and satellite glial cells (SGCs) *in vivo* and *in vitro*.

Results: Neuronal class III β-tubulin and non-phosphorylated neurofilaments are reliable markers of canine DRG neurons, which can be maintained over 18 days *in vitro*. Purification of neurons using Percoll centrifugation caused an enrichment of large over small neurons in DRG cell cultures. Canine SGCs show a coexpression of GFAP, CNPase, and vimentin, which are normally found in astrocytes, oligodendrocytes, and microglia. The frequent Sox2 expression of SGCs highlights their suggested stem cell properties.

Discussion (and/or Conclusions): The characteristics of canine DRG cell cultures are fundamental for their use in future research in different fields of neuroscience. Besides, DRGs may provide a source of cells with stem cell potency, which can easily be obtained and expanded and may be used for new therapeutic concepts of canine and human CNS diseases.

Notes:

P050 ANALYSIS OF CELLULAR PROLIFERATION IN LESIONS OF THE CANINE NASAL CAVITY

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Introduction: Nasal neoplasm and chronic inflammatory rhinitis are important causes of chronic nasal diseases and they account for approximately two-thirds of all dogs with persistent clinical signs from upper respiratory tract. Intranasal tumours comprise only about 2% of all neoplasms of dogs. Nearly two-thirds of tumours of this area are of epithelial origin. In majority, chronic rhinitis can be caused by well-known nasal diseases, such as foreign bodies. However, in over 25% of cases, the causes remain unknown and are being called idiopathic or non-specific rhinitis.

Material and methods: Tissue samples were collected from the nasal cavity during rhinoscopy and divided into two groups: nasal neoplasm and non-specific rhinitis. Ki-67 was investigated using the monoclonal mouse antibody. The Ki-67 labeling index was expressed as the number of Ki-67 positive nuclei per 1000 negative cells and by using Micro Image Program.

Results: The Ki-67 labeling index was significantly higher in non-specific rhinitis compared to neoplasm. The Ki-67 positive cells in rhinitis were observed in respiratory epithelium, in inflammatory cells in nasal mucosa and in stimulated lymph follicles. In nasal neoplasm Ki-67 positive cells were mostly neoplastic.

Conclusion: The present study demonstrates that cells proliferation is increased in non-specific rhinitis compared to nasal neoplasms. To our knowledge this is the first study evaluating expression of Ki-67 positive cells in canine nasal diseases.

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Poster Presentations ESVP/ECVP

P051 IBERIAN PIG PERI-IMPLANTATION PERIOD: UTERINE VEGF-VEGF RECEPTOR SYSTEM EXPRESSION

Notes:

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Introduction: The Iberian pig is an autochthonous breed from Iberian Peninsula known worldwide for the production of a cured-meat, the Iberian ham, and characterised by a low prolificacy. The aim of this study was to quantify the expression of the vascular endothelial growth factor (VEGF)-system molecules in endometrium during early pregnancy in pregnant Iberian sows.

Materials and Methods: Endometrium was collected from 8 sows at gestation day (gd) 17 and from 27 sows at gd 22. RT-qPCR was performed to detect mRNA expression of VEGF, VEGF receptor 1 (VEGFR1) and VEGF receptor 2 (VEGFR2). A Student's t-test was conducted to detect differences in VEGF-system expression (statistical significance $P < 0.05$).

Results: The mean (± standard error) values for expression of VEGF, VEGFR1 and VEGR2 were 64.80 (± 9.39); 34,66 (± 5.02) and 421.10 (±66.27), respectively, in endometrium from sows at gd 17, and 98.13 (± 11.52); 120.60 (± 8.68) and 158.10 (±21.23), respectively, in endometrium from sows at gd22. There were statistically significant differences between gd17 and gd22 for all three molecules ($P = 0.0333$, $P < 0.0001$ and $P = 0.0048$ for VEGF, VEGFR1 and VEGFR2, respectively), so that the expression of VEGF and VEGFR1 increased and the expression of VEGFR2 decreased as pregnancy advanced.

Discussion (and/or Conclusions): In endometrium from Iberian sows, mRNA expression of VEGF and VEGFR1 increases as pregnancy advances, whereas expression of VEGFR2 decreases, pointing out that the VEGF system may play an important role in the appropriate preparation of the endometrium for implantation.

P052 EXPRESSION OF VEGF-VEGFRS IN OVARIAN CORPORA LUTEA IN IBERIAN PIGS IN THE LUTEOLYTIC PHASE AND EARLY PREGNANCY

Notes:

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Introduction: The Iberian pig is an autochthonous Mediterranean breed characterised by a low prolificacy. The aim of this study was to quantify the expression of the vascular endothelial growth factor (VEGF)-system transcription in corpora lutea (CL) during early pregnancy and in non-pregnant Iberian sows and analyse the CVD (Capillary Vascular Density) in the subepithelial endometrium.

Materials and Methods: CL from 9 sows in late oestrus cycle and those from 15 sows in gestation day (gd) 17 were collected. RT-qPCR was performed to detect mRNA expression of VEGF, VEGFR1and VEGFR2. Quantification of CVD (positive blood capillaries in 5 HPF) was performed in CLs by immunohistochemistry against factor VIII (FVIII). A Student's t-test was conducted to detect differences in VEGF-system expression (statistical significance $P < 0.05$).

Results: The mean (± standard error) values for expression of VEGF, VEGFR1 and VEGR2 were 12.96 (± 2.29); 7.06 (± 1.29) and 245.50 (±85.64), respectively, in regressed CLs, and 87.49 (± 9.95); 21.41 (± 2.22) and 66.92 (±10.27), respectively, in CLs from sows at gd17. Early pregnancy CLs expressed significantly higher VEGF and VEGFR1 ($P < 0.0001$ for both) as compared to regressed CLs. FVIII mean CVD was higher in CL from sows at gd17 (16.33) as compared to regressed CLs (7.3).

Discussion (and/or Conclusions): Higher expression of VEGF and VEGFR1 is found in CL from Iberian sows at gd17 when compared to regressed CLs. These results highlight the importance of the VEGF-system molecules in the development and maintenance of the CL in pigs.

Poster Presentations ESVP/ECVP

P053 IMMUNOHISTOCHEMICAL MARKERS FOR THE ENTERIC NERVOUS SYSTEM OF THE COCKATIEL

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Introduction: A broad range of immunohistochemical markers for labelling components of the enteric nervous system is available for common laboratory animals (mice, rats), but the antigen expression profile of neurons and glia cells within gastrointestinal tissues of birds is largely unknown. The aim of this study is to establish a panel of immunohistochemical markers that are crossreactive with avian tissue and therefore suitable for investigations of the enteric nervous system (ENS) of the cockatiel.

Materials and Methods: Tissue samples from crop, proventriculus, gizzard and intestine as well as from brain, spinal cord, eye and peripheral nerves of 60 cockatiels were collected at necropsy, formalin-fixed and paraffin-embedded. Immunohistochemistry was performed employing the following neuronal and glial markers: neuronal class III β-tubulin, doublecortin, HuC/HuD, MAP2, nestin, NeuN, neurofilament, neuron specific enolase, periaxin, protein gene product 9.5 (PGP 9.5), glia fibrillary acidic protein (GFAP) and S-100.

Results: HuC/HuD, PGP 9.5 and neurofilament are useful markers for enteric neurons within all segments of the gastrointestinal tract of cockatiels, whereas antibodies against S-100 and GFAP can be successfully used for labelling intestinal glia cells. Antibodies recognising doublecortin, MAP2 and NeuN provide good labelling of neurons within the central nervous system, but fail to mark ENS. All other immunohistochemical markers did not mark neither central nor ENS in FFPE-material from cockatiels.

Discussion (and/or Conclusions): The panel of crossreactive markers provides a basis for further investigations of the structure and function of the enteric nervous system of cockatiels in health as well as in diseased state.

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P054 ANATOMY AND HISTOLOGY OF THE CIRCUMVENTRICULAR ORGANS IN ADULT CYNOMOLGUS MONKEYS (MACACA FASCICULARIS)

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Introduction: Circumventricular organs (CVOs) in the brain are characterised by extensive capillaries with fenestrated endothelial linings. Therefore their blood brain barrier is incomplete. While these CVO structures are relatively easy to find in a rat, it would take a concerted effort to find them in a brain of a Cynomolgus monkey, because NHP brains are more variable for trimming than dog and rodent brains.

Materials and Methods: Five brains of adult Cynomolgus monkeys were examined. Animals were from the stock and have not been in toxicological studies. The whole brains were preserved in 10% neutral buffered formalin, embedded in paraffin wax, sectioned serially at a nominal thickness of 5µm and stained with haematoxylin and eosin.

Results: In all mammals, the CVOs consist of 6 different neuroanatomical structures, known as (proceeding from rostral to caudal): 1. **vascular organ** of the lamina terminalis, 2. **subfornical organ** – present just beneath the fornix of the hippocampus, 3. **median eminence** – present along the floor of the ventral portion of the third ventricle, 4. **subcommissural organ** – immediately below the posterior commissure, 5. **pineal gland** – above or just posterior to the third ventricle, and 6. **area postrema** – at the posterior lip of the fourth ventricle. They are closely associated with neurons and can have sensory as well as secretory function. Several scientists also consider the choroid plexus a CVO, although it lacks direct association with neurons.

Discussion: Since the blood brain barrier of CVO's is incomplete, many systemically administered compounds gain access to the CVOs, making them an important target for safety evaluation. This presentation is intended to assist in the sectioning and identification of the CVOs in the brain of adult cynomolgus monkeys, and shall enable the toxicologic pathologist to detect any abnormal morphology in this widely neglected but functionally important neuroanatomical compartment.

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Poster Presentations ESVP/ECVP

P055 MESENCHYMAL STEM CELLS – PROMISING CANDIDATES FOR THE TREATMENT OF DEMYELINATING DISEASES?

Notes:

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Introduction: Mesenchymal stem cells (MSC) are considered to have regenerative functions in degenerative and inflammatory diseases. The aim of the study was to isolate canine MSC and to investigate, whether intra-ventricular MSC application has beneficial effects upon progression of chronic demyelination in Theiler's murine encephalomyelitis (TME).

Material and Methods: Cells were isolated from abdominal adipose tissue (cA-MSC) of a healthy beagle dog, characterised using flow cytometry (CD90, CD44, CD29) and tested upon their chondrogenic, osteogenic and adipogenic differentiation potential. Furthermore, cells or culture medium were transplanted into the lateral ventricle of SJL/JCrHsd mice at 7 or 42 days post TME virus (TMEV) infection (dpi). Clinic investigation and Rotarod tests were performed weekly. Animals were euthanised at 14, 49, 56 and 98dpi. Immunohistochemistry of cervical and thoracic spinal cord cross sections targeting CD44, MBP, TMEV, CD3 and CD107b was performed.

Results: *In vitro* >98% of cA-MSC expressed CD90, CD44, CD29 and cultivation in induction media resulted in a differentiation into the above mentioned lineages. RotaRod investigation revealed no significant differences between TMEV-infected groups. CD44 positive cells were not detected in the spinal cord. CD3 positive cells in TMEV-infected cA-MSC transplanted animals were significantly increased or decreased compared with medium transplanted animals at 14 or 98dpi, respectively. Demyelination, number of TMEV- and CD107b-positive cells showed no significant differences between these groups.

Conclusion: In summary MSC can be isolated from canine adipose tissue. Following heterologous transplantation cA-MSC failed to integrate into the host tissue and to ameliorate progressive demyelination in TME.

P056 MDM2 AND CDK4 EXPRESSION IN CANINE LIPOSARCOMA

Notes:

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Introduction: Canine liposarcoma (LP) is an uncommon neoplasm usually arising in the subcutis and classified as well-differentiated (WDLP), myxoid (MLP) and pleomorphic (PLP). In man, LP is classified as WDLP/atypical lipomatous tumour (ATL), dedifferentiated (DDLP), MLP and PLP. WDLP/ALT and DDLP are considered different morphological presentations of the same entity, bearing the amplification of genes encoding for MDM2 and CDK4, and overexpressing these proteins. The aim of this study is to assess the expression of MDM2 and CDK4 in canine LP by immunohistochemistry (IHC).

Materials and Methods: Selected cases were classified according with the WHO classification of tumours of domestic animals. When this was not possible the human classification was applied. Cross reactivity of antibodies anti-mdm2 and CDK4 was assessed by western blot analysis. Immunohistochemistry was performed of formalin fixed tissue applying a heat-induced antigen retrieval. Cases were considered positive when more than 10% of neoplastic cells had nuclear staining.

Results: Forty-seven LP were collected: 19 WDLP, 18 PLP, and 7 MLP. Three cases were consistent with DDLP. Fifteen cases expressed MDM2 and 37 CDK4. MDM2 and CDK4 were respectively expressed in 12/19 and 17/19 cases WDLP, 1/7 and 5/7 MLP, 0/18 and 12/18 PLP, 2/3 and 3/3 DDLP. Taken together WDLPs and DDLPs expressed mdm2 in 14/22 (63.6%) and CDK4 in 20/22 cases (90.9%).

Discussion (and/or Conclusions): These results suggest that WDLP/DDLP may represent a biological entity characterised by MDM2 and CDK4 overexpression, paralleling LP in man, and suggesting the presence of similar gene amplifications.

Poster Presentations ESVP/ECVP

P057 THE COMPARISON OF SURVIVIN EXPRESSION IN CANINE AND HUMAN SPONTANEOUS OSTEOSARCOMA AND ESTABLISHED OSTEOSARCOMA CELL LINES

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Introduction: Survivin is a protein belonging to the family of inhibitors of apoptosis (IAP). This protein is expressed by some normal cell types, and its overexpression occurs in many types of cancers. The aim of the study is to compare the expression of Survivin in canine and human spontaneous osteosarcoma with expression in cells derived from the established canine (D-17) and human (U-2 OS) osteosarcoma cell line.

Materials and Methods: Paraffin blocks from the archival collections (10 samples of canine and 5 samples of human osteosarcoma) were cut into thick fragments of 4µm. Cells from human and canine osteosarcoma cell line were imposed on the 10-well hydrophobic slide (Thermo Scientific). Immunohistochemistry was performed using Survivin antibodies (clone 12C4, DAKO).

Results: The strength of Survivin expression in canine and human spontaneous osteosarcoma was at the level of+ +, whereas in the case of the canine osteosarcoma cell line at the level of + +, and the human osteosarcoma cell line at the level of +.

Discussion (and/or Conclusions): Analysing the obtained results, it can be seen that the expression of Survivin was at the comparable level in both spontaneous osteosarcomas and the canine cell line while weaker in the human cell line. This may indicate that the studied tumours and cells from cell lines demonstrate the comparable level of resistance to apoptosis-inducing agents. In vitro studies and obtained results may be useful in refining a suitable animal model that can be used in research on human oncogenesis.

Notes:

P058 THE COMPARISON OF N-CADHERIN EXPRESSION IN THE CANINE AND HUMAN OSTEOSARCOMA CELL LINE AND IN SPONTANEOUS OSTEOSARCOMAS

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Introduction: N-cadherin is a protein that plays an important role in intercellular adhesion. The expression level of this protein in the tumour may be useful in assessing the ability of the tumour to metastasize. The aim of the study is to compare the expression of N-cadherin in the cells of canine and human spontaneous osteosarcoma with the expression of this protein in the cells derived from the established canine (D-17) and human (U-2 OS) osteosarcoma cell lines.

Materials and Methods: For testing were used 10 samples of canine osteosarcoma and 5 samples of human osteosarcoma. Cells from cell line of human and canine osteosarcoma were imposed on the 10-well hydrophobic slide. Immunohistochemistry was performed using N-cadherin antibodies (Clone 6G11, DAKO).

Results: The expression of N-cadherin according to Remmel scale in the cells of spontaneous canine osteosarcoma was assessed at 4 points, and for human osteosarcoma at 3 points, while in the canine osteosarcoma cell line it was at 12 points, and in the human was at 8 points.

Discussion (and/or Conclusions): The decreased expression of N-cadherin in the spontaneous osteosarcomas compared with their cell lines may provide their relatively high metastatic potential. The stronger expression of the tested protein in the cell lines may be due to the fact that these cells may have stronger adhesion when compared to spontaneous tumours. It was also shown that the decreased expression of N-cadherin in human osteosarcoma, when compared to canine osteosarcoma, may indicate the greater potential of the cancer for generating metastatic lesions in humans.

Notes:

Poster Presentations ESVP/ECVP

P059 THE INFLUENCE *IN VITRO* OF RISEDRONATE SODIUM ON THE CANINE (D-17) AND HUMAN (U-2 OS) OSTEOSARCOMA CELL LINE

Notes:

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Introduction: Risedronate sodium is a new generation agent belonging to bisphosphonates, drugs used both in human medicine and veterinary medicine in the treatment of, among others osteoporosis, Paget's disease and multiple myeloma. Bisphosphonates have also cytotoxic activity and are used as pre-medication in the treatment of osteosarcoma. The aim of the study is to evaluate the viability of the canine and human osteosarcoma cell line treated with risedronate sodium dependent on the concentrations applied.

Materials and Methods: The tested cell lines were exposed to risedronate sodium for a period of 72 hours. The drug was tested in the following concentrations: 300,150,100, 30, 15, 10, 3, 1.5, 1, 0.3, 0.15**µ**g/ml. The viability of the cells treated with the tested substances was evaluated using the MTT assay. Four independent repetitions were performed, then for each EC50 (half maximal effective concentration) were calculated, and the results are given as the average of these values.

Results: The EC50 value of risedronate sodium in the canine osteosarcoma line was 144,83±6,22 µg/ml, and in the human osteosarcoma line 98,1±5,4 µg/ml. For the concentration of 300 **µ**g/ml the percentage of live cells in the case of the canine osteosarcoma cell line was 29.1±2.17%, while in the case of the human osteosarcoma cell line was 14.84±2.36%.

Discussion (and/or Conclusions): The results indicate that canine osteosarcoma cells are more resistant to risedronate sodium than human osteosarcoma cells. It allows to hope for extending the research and the introduction of risedronate sodium to standard therapy of osteosarcoma in both animals and humans.

P060 THE INFLUENCE IN VITRO OF ETOPOSIDE AND MELOXICAM PER SE AND CONCURRENTLY ON THE CANINE (D-17) AND HUMAN (U-2 OS) OSTEOSARCOMA CELL LINE

Notes:

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Introduction: Etoposide is a cytostatic drug which is used in treating some types of lymphoma. Meloxicam is a nonsteroidal anti-inflammatory drug which has also a cytotoxic effect. The aim of the study is to assess the viability of the cells of canine and human osteosarcoma cell line treated with etoposide and meloxicam *per se*, and concurrently.

Materials and Methods: The cells lines were exposed to the tested drugs *per se* and concurrently for a period of 72 hours. The viability was evaluated using the MTT assay. Four independent repetitions were performed and the results are given as the average of these values, whereas for etoposide and meloxicam EC50 values (half maximal effective concentration) were calculated.

Results: The EC50 values of etoposide and meloxicam in the canine line were 6,27±0,31 µg/ml and 149,94±9,17 µg/ml respectively, and in the human line 2,72±0,51µg/ml and 234,02±5,96 µg/ml respectively. For example, for the concentration of etoposide at 0.1 µg/ml, meloxicam at 100 µg /ml and the combination of these concentrations the percentage of live cells in the canine line amounted to 89.6±4.2%; 83.4±3.4% and 51.45±4.57%, while in the human line resulted in 93.98±4.13%; 57.54±5.49% and 73.78±2.79% respectively.

Discussion (and/or Conclusions): Analysing the obtained results it can be noted that canine cells are more sensitive to meloxicam than human cells; in the case of etoposide reverse reactions were observed. The most interesting is the presence of synergism between etoposide and meloxicam which can be useful in the development of new anti-osteosarcoma therapy schedules in both animals and humans.

Poster Presentations ESVP/ECVP

P061 CHARACTERISATION OF AMYLOID IN EQUINE RECURRENT UVEITIS AS AA AMYLOID

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Introduction: Equine recurrent uveitis (ERU) is a leading cause of blindness in horses worldwide and is the only spontaneously occurring animal model of human immune mediated uveitis. Deposition of amyloid-like material along the non-pigmented ciliary epithelium (NPCE) is reported to be a specific finding in ERU and is diagnostic for this condition. However, the nature of the amyloid deposits is unknown. The purpose of this report is to describe two horses with histopathological changes consistent with ERU and ciliary body amyloid deposits consisting of AA amyloid.

Materials and Methods: Ciliary body tissue from two horses with chronic uveitis and histological lesions consistent with ERU were examined by light microscopy and immunohistochemistry for AA amyloid, CD3 and CD20 was performed. Samples for mass spectrometry were collected by laser microdissection of Congo red stained sections from formalin fixed paraffin embedded tissues. Transmission electron microscopy was used for examination for ciliary body from one of case.

Results: Microscopical findings in the ciliary body included deposits of amyloid lining the non-pigmented epithelium, intracytoplasmic, rod-shaped, eosinophilic inclusions and intraepithelial T-lymphocyte infiltrates. Ultrastructural examination of the ciliary body of one horse confirmed the presence of abundant extracellular deposits of non-branching fibrils ranging from 9 –11 nm in diameter consistent with amyloid. Immunohistochemistry of amyloid revealed strong positive labeling for AA amyloid, and mass spectrometry showed amyloid to consist primarily of SAA1, in both cases.

Discussion (and/or Conclusions): The current findings suggest that a localised, intraocular AA amyloidosis may occur in horses with ERU.

Notes:

P062 DOMINANT EXPRESSION OF INTERLEUKIN-8 VERSUS INTERLEUKIN-1B, AND TUMOUR NECROSIS FACTOR ALPHA, IN LUNG OF LAMBS EXPERIMENTALLY INFECTED WITH *MANNHEIMIA HAEMOLYTICA*

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Introduction: The immunohistochemical expression and the lung extracts concentrations of Interleukin-1 Beta (IL-1β), Tumour Necrosis Factor Alpha (TNFα) and Interleukin-8 (IL-8) in the lung of lambs experimentally infected with *Mannheimia haemolytica* were investigated.

Materials and Methods: The lambs were randomly assigned to 2 groups: infected and uninfected controls. The inoculum in each lambs of the infected group was 1.5×10^9 colony-forming units of *Mannheimia haemolytica (Mh)* in 5 mL sterile nutrient broth. The control lambs were inoculated with 5 mL of sterile nutrient broth. The control and infected animals were killed from 1 to 15 days post-infection (dpi).

Results: These findings demonstrate a temporal association between pulmonary expression of these cytokines and lung pathology in ovine pulmonary pasteurellosis. Given that the lung expression of IL-8 was much greater than that of TNFα and IL-1β, the anti-cytokine agents directed at this mediator could be more useful in the prevention and treatment of this disease.

Conclusions: The results of this study suggest that IL-1β, TNF-α and IL-8 inflammatory cytokines may play an important role in enhancing the biological response of *Mh* and contribute to the development of the lung lesions in ovine pulmonary pasteurellosis. Our findings indicate that IL-8 is the dominant inflammatory cytokine expressed within the lungs in ovine pasteurellosis, accordingly anti-cytokine agents targeting this mediator may be most useful in the prevention and treatment of this disease, provided as a complementary measure to combat the causative pathogen agent, together with prophylactic measures to prevent the infections.

Notes:

Poster Presentations ESVP/ECVP

P063 CHEMOKINES AND INFLAMMATION IN NEONATAL OVINE LUNG FOLLOWING EXPERIMENTAL INOCULATION OF *BOVINE RESPIRATORY SYNCYTIAL VIRUS*

Notes:

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Introduction: This study sought to determine the immunohistochemical expression of interleukin-1 beta (IL-1β), tumour necrosis factor alpha (TNFα), interferon gamma (INFγ), IL- 4, IL-6, IL-8, IL-10 and IL-12 and to measure the levels of these cytokines in lung tissue from lambs experimentally infected with BRSV.

Materials and Methods: Lambs ($n = 15$) were inoculated at 2 days of age with 20 mL of viral inoculum ($1.26x10^6$ $TCID_{50}$ per mL) or sterile media ($n = 15$). Vital signs (rectal temperature, pulse and respiratory rates) were monitored daily in control and infected lambs. Lambs were euthanatised and necropsied at 1, 3, 5, 7 and 15 days post inoculation.

Results: Findings demonstrated a temporal association between pulmonary expression of these cytokines and lung pathology in BRSV-infected lambs. 1.- IL-4 and IL-10 are not primarily involved in the pathogenesis of BRSV infection in neonatal lambs; 2.- A significant increase of IL-1β, TNFα, INFγ and IL-6 proteins and labeled cells was found suggesting that these cytokines may play a major role in enhancing the biological response to BRSV, contributing to the development of lung lesions in BRSV-infected lambs. 3.- A significant increase of concentrations and number of immunolabeled cells of IL-8 and IL-12 was observed in infected lamb lungs throughout the study.

Conclusions: Given the marked induction of IL-8 and IL-12, anti-cytokine agents targeting these inflammatory cytokines may be useful in the prevention and treatment of BRSV, in conjunction with measures to combat the causative pathogen and prophylactic methods aimed at preventing infection.

P064 PRIMARY IRRITANT CONTACT DERMATITIS IN SHEEP

Notes:

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INTRODUCTION: Contact dermatitis in sheep caused by accidental application of chemical compounds is uncommon and irritant or allergic contact dermatitis in farm animals underreported.

MATERIALS AND METHODS: Forty-two pregnant ewes from a sheep flock of 120 Lacaune breed ewes of various ages, presented sudden onset of severe dermatitis. The cause was suspected to be a false dilution of commercial bleach, which had been added to a disinfectant solution and spread on the floor to prevent environmental infections. Macroscopic lesions observed were severe erythema and oedema in hairless regions such as ventrum – lower abdomen, mammary skin, inguinal, axillary regions – and in some animals the face, lips and periorbital regions. From 10 animals (7 diseased and 3 controls) skin punch biopsy samples were obtained.

RESULTS: Histopathology revealed moderate to marked oedema, intense perivascular and mild periadnexal mainly mononuclear dermatitis and capillary dilatation at the superficial dermis. The epidermis showed focal orthokeratotic hyperkeratosis and intracellular oedema. Immunohistochemistry for T-cell populations in the skin revealed the majority as CD3+.

DISCUSSION: Histopathology of irritant contact dermatitis depends on various parameters, playing role in the formation of lesions, such as nature of the irritant compound(s) and consequently their mode of action, duration of their contact with the skin and their concentration, ways of penetration and individual response of skin to these chemicals. Contact dermatitis to sodium hypochlorite bleach is rarely reported, despite its frequent use. The irritancy of aqueous chlorine is presumably the cumulative effect of diverse agents used in different manufactured compositions.

Poster Presentations ESVP/ECVP

P065 SPONTANEOUS HASHIMOTO-LIKE THYROIDITIS IN MALE B.U.T. 6 TURKEYS

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Introduction: In the context of a study on the tolerance of rapeseed meal (RSM) in B.U.T. 6 turkeys, thyroid glands were histologically and immunohistochemically examined because of potential thyrostatic effects.

Materials and Methods: The turkeys were separated in five groups based on the amount of rapeseed in the food. Thyroid glands (Control 1: no rapeseed, without coccidiostats n=12; Control 2: no rapeseed, with coccidiostats n=8; RSM-1 n=12, RSM-2 n=12, and RSM-3 n=12; RSM: different levels of rapeseed, without coccidiostats) were dissected after slaughter, formalin fixed and processed routinely for HE staining. Sections were examined and given an estimated score of severity of lymphocytic thyroiditis. CD3 and Pax-5 expressing cells were immunohistochemically detected and semiquantified on representative slides.

Results: Irrespectively of the RSM feeding, in all groups, there was a high incidence of chronic, lymphocytic thyroiditis, showing scattered foci (n=33 animals), mild (n= 9), moderate (n= 3) and severe (n= 5) lymphocytic infiltrations. Thus, 14% of examined thyroids showed a moderate to severe lymphocytic thyroiditis. The inflammatory infiltrate was characterised by predominantly T cells admixed with scattered B cells, and germinal centre development, independent of the lesion score.

Discussion: Thyroiditis appears to be a common, spontaneously occurring disease in the strain of turkeys investigated here, unrelated to the study goal. The features of thyroiditis found here resemble Hashimoto's disease which is an autoimmune disorder in humans, non-human primates, dogs, Buffalo rats and Obese strain of chickens. Future studies should aim at elucidating the functional and metabolic significance in affected turkeys.

Notes:

P066 POLARISATION OF MACROPHAGES/MICROGLIA IN THEILER'S MURINE ENCEPHALOMYELITIS

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Introduction: Macrophages/microglia (M/M) play an important role for antigen presentation and T cell activation in Theiler's murine encephalomyelitis (TME). So far, disease phase specific changes of the polarity of M/M have not been investigated in detail in this model for demyelinating disorders.

Materials and Methods: In order to determine spatiotemporal changes of pro-inflammatory M1-type and anti-inflammatory M2-type M/M, Theilervirus-infected SJL-mice were euthanised at 14, 42, 98 and 196 days post infection (dpi) and the spinal cord was investigated by histology, immunofluorescence and microarray analyses.

Results: Increasing numbers of $CD68^+$ and $MAC3^+$ M/M were found with disease progression. Arginase-1^+ M2-type cells significantly increased between 42 and 98 dpi, while a significant increase of $CD16/32^+$ M1-type cells was observed at 42, 98, and 196 dpi. Microarray analyses revealed a differential expression of 59 polarisation-specific genes with a dominance of M1-associated genes during the early demyelinating phase.

Discussion: Findings of the present study support the hypothesis that an imbalance between M1-type cells and neuroprotective M2-type cells represents a prerequisite for immune mediated processes and subsequent demyelination in TME. In addition, prolonged M1-polarisation might contribute to viral persistence in susceptible mouse strains.

Notes:

Poster Presentations ESVP/ECVP

P067 IN SITU EVALUATION OF THE PORCINE GENITAL TRACT INFECTED WITH *CHLAMYDIA TRACHOMATIS*; COMPARATIVE EVALUATION OF IMMUNISED AND NON-IMMUNISED GÖTTINGEN MINIPIGS

Notes:

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Introduction: Vaccine candidates against the sexually transmitted bacterium *Chlamydia trachomatis* are being tested with focus on efficacy against bacterial replication. Large animal models in this area are rare, and little focus has been on the immune cell infiltration in challenged animals with different immunological status. We have investigated the histopathological changes in immunised and non-immunised minipigs genitally challenged with *Chlamydia trachomatis*.

Materials and Methods: Sexually mature Göttingen minipigs were intramuscularly immunised with whole inactivated *C. trachomatis* formulated in adjuvant (CAF01) (n=8). A control group was given adjuvant only (n=9). All pigs were vaginally inoculated with bacteria and monitored by vaginal swabs for cultivation and antibody detection. Pigs were euthanised 12 days after infection, and the genital tract evaluated by histopathology and immunohistochemically.

Results: Pigs in the immunised group were significantly protected compared to the control group on day 3 following infection, after which the groups were equally protected. In the lower part of the genital tract, an increased cellular infiltration of lymphocyte-like cells (CD3+) and plasma cells was noted in the immunised pigs, and lymphoid follicles were noted. Vaginal antibodies on the mucosal surface were increased short after infection in the immunised group.

Conclusions: An immunisation elicited a cell-mediated and humoral immune response, which after genital challenge gave rise to an increased immune cell infiltration at the site of infection. Although the naïve animals also quickly clear the infection, the preexisting immune response and its recruitment of cells to the genital tract seem to make a difference in protection early in infection.

P068 A CASE OF CHRONIC PANCREATITIS IN A HORSE

Notes:

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Introduction: Chronic pancreatitis is a sporadically occurring disease in horses. Ascending intestinal infections and intraductal parasites are discussed as a possible cause.

Materials and Methods: A 16 year-old Friesian gelding with a history of relapsing colic was submitted for necropsy. Organ samples were fixed in 10% buffered formalin, embedded in paraffin and plastic, sectioned and stained by haematoxylin and eosin, Giemsa and Masson Trichrom.

Results: Macroscopically, a firm, solid, 20 x 8 x 8 cm mass was found in the pancreatic area. The cut surface had a lobular structure with multiple cavities. Microscopically, a severe chronic diffuse fibrosing pancreatitis with acinar-ductal metaplasia and duct dysplasia was diagnosed.

Discussion: In horses with relapsing colic, chronic pancreatitis must be considered as a possible differential diagnosis. Acinar-ductal metaplasia and ductal dysplasia are considered to be precursor lesions of murine and human pancreatic ductal adenocarcinoma.

Poster Presentations ESVP/ECVP

P069 ENDOMETRIAL CYTOLOGY IN MARES: EFFECTIVENESS AND CORRELATION WITH BACTERIOLOGY

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Introduction: The detection of subclinical endometritis in equine breeding industry is a frequently overlooked and often underdiagnosed problem resulting in an important prerequisite for chronic infertility of mares. The purpose of the study was to assess whether cytology can be validated as a quick and effective tool to diagnose subclinical endometritis in correlation with bacteriological examinations.

Materials and Methods: 73 uterine smears from 63 mares were collected by uterine flush technique and after centrifugation, examined by light microscopy (100x and 400x magnification). According to the number of neutrophils (PMNs), endometritis was graded as absent or mild (grade 1), moderate (grade 2) or severe (grade 3). An aliquot of the fluid was plated out on blood agar, McConkey agar and Sabouraud's agar and incubated aerobically at 37 °C for 24-48 hours. Statistical analyses were performed.

Results: 38 of 73 (52%) smears were positive for endometritis of different degrees of severity (18% grade 1; 55% grade 2 and 27% grade 3). The statistical correlation between cytology and bacteriological tests was significant ($p = 0.0016$), and appeared to be strongly correlated with the isolation of *Streptococcus equi zooepidemicus* ($p<0.0001$). No statistical correlation was found with isolation of *Escherichia coli*.

Discussion (and/or Conclusions): The study confirms the uterine cytology as the gold standard in the diagnosis of subclinical endometritis of the mare and emphasises its correlation with bacteriological examination. This has proved particularly effective in identifying S. *equi zooepidemicus* infections and less effective for E. *coli* infections probably due to the different pathogenic mechanism.

Notes:

P070 AORTIC VALVE PATHOLOGY IN THE EUROPEAN BISON *(BISON BONASUS)* – A CASE REPORT

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Introduction: The available literature provides no descriptions of the aortic valve pathology of the European bison although comparable pathology has been more fully studied in people.

Material: The study was performed on 26 hearts of adult European bisons *(Bison bonasus)* of various ages and both sexes. The observations were carried out with the use of an operating microscope with integrated video channel.

Results: In 25 studied bisons the aortic valve consisted of the three semilunar leaflets: left, right and septal (*valvula semilunaris sinistra, dextra et septalis*). The free edges of the adjacent semilunar leaflets of the aortic valve were getting more closely adjoined towards the wall of aorta, forming an almost unified commissure. Three aortic valve commissure were noticed: left, right and intermediary (*commissura valvae aortae sinistra, dextra et intermedia*). In one case (male, 3.5 years old, 390 kg body weight) perforations of the left and septal semilunar leaflets were observed. Pathological changes were localised on both size of the left aortic valvae commissure. The lateral fibrous attachments of the leaflets were well developed.

Discussion and conclusion: The aortic valve can be affected by a range of diseases such as rheumatic valve disease, degenerative disease or endocarditis. Perforation of the semilunar leaflets causes blood to flow in the reverse direction during ventricular diastole, from the aorta into the left ventricle.

Notes:

Poster Presentations ESVP/ECVP

P071 PATHOLOGICAL CHANGES OF COLON IN THE TNBS MODEL OF COLITIS IN RATS TREATED WITH FUNCTIONAL FOOD CONTAINING PROBIOTIC/SYNBIOTIC FORMULATION

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Introduction: Lactic acid bacteria may have a preventive role in inflammatory bowel diseases due to inappropriate and continuing inflammatory response to commensal microbes in a genetically susceptible host. The aim of this study was to evaluate the histopathological changes of the colon in rats with chemically induced colitis, after oral administration of functional food product containing non-encapsulated and encapsulated probiotic/synbiotic.

Materials and Methods: Three groups of female Wistar rats (n=6, 180-250 g, 10-14 weeks old) which TNBS (trinitrobenzene-sulphonic acid) colitis was induced, ayran containing free probiotic/synbiotic and encapsulated synbiotic, respectively, was administered orally, once daily (8.5-8.9 log cfu/g of the food product) and control group treated with drinking water and plain ayran were used. Histopathological examination was performed on colon segments that were fixed in 10% (v/v) formalin, embedded in paraffin and 3-5 μm sections were stained with haematoxylin-eosin.

Results: Histopathological examination of the colons of rats receiving ayran containing synbiotic microparticles showed significant differences compared with the control groups. Namely, ulcerations on mucosa and sub-mucosa, accompanied by extensive inflammatory infiltrate and congested blood vessels, were observed in the non-treated group, whereas higher integrity of mucosal architecture of colon tissue was apparent when encapsulated synbiotic was administered.

Discussion (and/or Conclusions): Histopathological examination of colon has shown that ayran containing microencapsulated synbiotic is a convenient anti-inflammatory therapy. These results are confirmed with biochemical examination of myeloperoxidase activity.

Notes:

P072 THE INDUCTION OF CHRONIC ULCERATIVE COLITIS IN RATS – OBTAINING AN ANIMAL MODEL FOR TESTING THE EFFICACY OF DRUGS

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Introduction: In the search for drugs used in the treatment of gastrointestinal inflammation in human, animal models are used. In these models, intestinal lesions, which are similar to human Inflammatory Bowel Disease (IBD), are induced.

Materials and Methods: The experiment was conducted on 80 female Wistar rats. The animals were randomised into 4 groups, i.e. A, B, C, and D depending on elimination day. In each group, 5 females received rectally 50% ethyl alcohol, and ethanolic TNBS (2, 4, 6-trinitrobenzene sulfonic acid) at the doses of 12.5 mg / kg, 25 mg / kg, and 50 mg / kg body mass. Group A was euthanised after 28 days, group B after 21 days, group C after 14 days, and group D after 7 days. During dissection, a distal part of the colon of each animal was collected. After the macroscopic evaluation, tissues were fixed in a 4% neutral buffered formaldehyde solution, dehydrated, embedded in paraffin, and stained with HE.

Results: The autopsy revealed hyperaemia, erosions, and ulceration in the colon wall. Adhesions of the bowel wall with adjacent organs were also observed. Histopathology additionally showed fine-cell infiltration in each layer of the colon wall, stimulation of the lymphoid nodules, and mucosal regenerative processes. The incidence of these lesions decreases over time starting from exposure, but increases with increasing doses of TNBS.

Conclusions: The use of TNBS-alcoholic solutions in the induction of chronic ulcerative colitis in rats is an inexpensive and technically simple method for obtaining an animal model.

Notes:

Poster Presentations ESVP/ECVP

P073 A NEW WAY OF PROBIOTIC BACTERIA DELIVERY: CLINICO-PATHOLOGICAL EVIDENCES IN A MURINE MODEL

Notes:

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Introduction: Health-promoting benefits of probiotics have been demonstrated in gastrointestinal domain after oral administration. Evidences show that probiotics communicate with the host by modulating key signaling pathways in gastrointestinal lymphoid associated tissue (G.A.L.T.). In order to understand how probiotic agents act within deep respiratory tract, we have developed a mouse model of respiratory delivery, in attempt to examine the effects of *Lactobacillus brevis* (strain Cd2 Dsm 11988) administration in the pulmonary environment, in terms of host responses, survival rate, modifications of resident microbiota, gross- and histological modifications.

Materials and Methods: Thirty CD1-male mice were exposed daily, to different dosage of aerosolised *Lactobacillus brevis*, for five days. After treatment, mice were euthanised and airways, lungs and bronchoalveolar lavage fluid (B.A.L.F.) were collected and processed for histopathological studies, and microbiota analysis. We analysed mucosal modification and cell response in lungs through histological and immunohistochemical evaluations, using several antibodies to immunophenotyping the immunitary response.

Results: Respiratory delivery of probiotic bacteria was well tolerated in mice, resulting in mild to absent inflammatory reaction in airways mucosa and lung's parenchyma. A considerable hyperplasia of the bronchial-associated lymphoid tissue (B.A.L.T.) and an increased number of pulmonary intravascular and alveolar macrophages was also documented. Analysis of microbioma showed an interesting modulation of respiratory tract-resident bacterial populations.

Discussion (and/or Conclusions): Our newly murine model of probiotic delivery may be useful in attempt to positively modulate the airways/lung response to different pathogens. Our results suggest that probiotic can be used as a novel therapeutic or preventative strategy to manage respiratory diseases.

P074 EFFECTS OF PHYSICAL EXERCISE ON *GASTROCNEMIUS* MUSCLE IN A RAT MODEL OF MAMMARY CANCER

Notes:

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Introduction: Mammary cancer is one of the most frequent cancers worldwide. Ultrasonography has been frequently used to evaluate several body tissues. The aim of this study was to evaluate the effects of physical exercise on *gastrocnemius* muscle in a recognised rat model of mammary cancer chemically-induced by *N*-methyl-*N*-nitrosourea (MNU).

Materials and Methods: Animal procedures were approved by the Portuguese DGAV (no.008961). Female Sprague-Dawley rats were divided into two groups: sedentary (n=11) and exercised (n=10). At seven weeks of age, the animals received an intraperitoneal injection of MNU (50mg/Kg). Animals from exercised group performed endurance training on a treadmill running during 34 weeks. *Gastrocnemius* muscle was palpated for tonus evaluation and was examined by ultrasonography. At necropsy, the muscle was collected and weighed. Myostatin serum levels were assessed by immunoblotting.

Results: All animals developed mammary tumours. The weight, length, width and echogenicity of *gastrocnemius* muscle from exercised animals was lower than in sedentary animals (p>0.05). The tone of muscle was higher in exercised animals. Myostatin levels were higher in exercised animals than in sedentary animals (p>0.05).

Discussion and Conclusions: The muscle weight agrees with the width and length measured by ultrasonography. The higher tonus is associated with a greater development of muscle fibers in exercised animals, associated with a reduction of the echogenicity that reflects the reduction of fat infiltration in these animals. High levels of myostatin have been described in conditions associated with muscle wasting, namely cancer. Muscle ultrasonography is an important tool to identify changes in muscle structure.

Poster Presentations ESVP/ECVP

P075 PEQUI (*Caryocar brasiliense*) EXTRACT ALTERS MITOGEN REGULATED KINASES ACTIVATION ON BRAIN ISCHAEMIA AND REPERFUSION INJURY IN RATS FED A HIGH-CALORIE DIET

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Introduction: Pequi (*Caryocar brasiliense*) is an edible fruit from "Cerrado", a savanna-like ecosystem in central Brazil. Ethanol extracts (EEP) of pequi epicarp and external mesocarp have shown antioxidant effects *in vitro*, and possible therapeutic activity of oxidative stress-related diseases. Since Mitogen Activated Kinases (MAPK) Phospho-p44/42 (ERK1/2) and Phospho-p38 are activated by oxidative stress, we verified the EEP effect on expression of their phosphorylated (active) forms on induced cerebral ischemia and reperfusion in rats fed high-calorie diet.

Materials and Methods: Rats (n=24) were randomly divided into 4 groups: regular diet (RE) or high-calorie diet (HE) groups supplemented daily with 600 mg/kg of EEP for 45 days, and control groups on regular (RC) or high-calorie diet (HC) without supplementation. After 30-minute cerebral ischaemia and a 24 hour-reperfusion period, euthanasia was performed and brains were harvested for H&E and immunohistochemical evaluation of the expression of phosphorylated forms of ERK1/2 (Thr202/Tyr204) and p38 (Thr180/Tyr182).

Results: Ischaemic lesion area was larger in untreated (RC and HC) groups. HC showed significantly ($p<0.05$) higher levels of phospho-ERK1/2 as compared to all other groups, including EEP-treated. Phospho-p38 levels were not significantly different ($p>0.05$) between groups.

Discussion/Conclusions: ERK1/2 activation plays important role in cell injury and death. While high-calorie diet alone induced significantly higher levels of ERK 1/2 activation, HE rats showed similar activation patterns of RE and RC animals. Also, neuronal death was more conspicuous in rats not supplemented with EEP. These findings point to a possible neuroregulatory effect of EEP in this model of cerebral ischaemia-reperfusion injury.

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P076 ALLERGIC CONTACT DERMATITIS IN MICE IS NOT AFFECTED OR PENETRATED BY AHAPS-FUNCTIONALISED SILICA NANOPARTICLES

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Introduction: Nanoparticles (NP) are widely used in every-day products as well as biomedical applications and intended or unintended contacts between NP and the skin are likely. It is still unclear, however, whether preexisting skin diseases may predispose for unwanted NP penetration or whether NP may even aggravate hypersensitivity reactions. The aim of our study was to investigate whether AHAPS-functionalised silica NP penetrate through inflamed skin or affect the course of allergic contact dermatitis (ACD).

Material and Methods: SKH1 mice (n= 5 per group) with oxazolone-induced ACD were topically treated with 250 µg silica NP of 55 ± 6 nm in diameter and N-(6-aminohexyl)(3-aminopropyl) trimethoxysilane (AHAPS)-functionalisation or ultra pure water for five consecutive days. Mice were examined clinically, histopathologically and in several molecular parameters. NP were visualised by fluorescence as well as transmission electron microscopy.

Results: The AHAPS-silica NP were only identified in the superficial layers of the stratum corneum of the epidermis but not in deeper epidermal or dermal layers, despite severe inflammation and barrier disruption. Furthermore, the NP did not affect the course of ACD.

Conclusion: In contrast to previous general apprehensions on NP and experimental data on other NP, AHAPS-functionalised silica NP seem not to penetrate through inflamed skin or affect the course of ACD in this model. Whether they may serve as suitable drug carriers in different skin conditions remains to be studied.

Notes:

Poster Presentations ESVP/ECVP

P077 PREPARATION AND ASSESSMENT OF BIOCHEMICAL AND HISTOPATHOLOGICAL PROPERTIES OF EXTRACELLULAR MATRIX PRODUCED FROM HOST TISSUE RESPONSE AGAINST SUBCUTANEOUSLY-IMPLANTED DEXTRAN HYDROGEL IN RATS

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Introduction: Tissue Engineering is an interdisciplinary science that is based on the use of three-dimensional scaffolds for tissue formation in order to produce artificial limbs or repair injuries which are irreversible. These scaffolds used as substrates for cell adhesion and migration, proliferation and cell differentiation. Extracellular Matrix (ECM) is responsible for proliferation conduction, cell adhesion and migration.

Materials and Methods: 12 patches of synthetic dextran hydrogel scaffolds got prepared. These, were implanted under the skin of 6 white rats. After 2 months, the patches, which were surrounded with a host tissue response (the membrane), were harvested. By using mechanical and chemical methods, the membrane was decellularised. Selected parameters: tissue DNA content by flow cytometry, levels of sulfated glycosaminoglycan (GAGs) with a spectrophotometer, and the status of histological sections of tissues and cells, were investigated before and after the decellularisation procedure.

Results: Histopathological findings revealed, in the host tissue response, there is not any inflammatory reaction. The amount of DNA content by flow cytometry before and after decellularisation demonstrated approximate success in decellularisation method. Evaluation of sulfated glycans expressed high levels of these substances in treated tissue.

Discussion: As this ECM was driven from healthy tissue, it can promote different stages of migration, proliferation and cell differentiation, in comparison with synthetic scaffolds. The results revealed that the treated tissue is suitable for using in xenogenic and allogenic grafts.

Notes:

P078 MICROSCOPIC EVALUATION OF THE CORNEA IN THE ISOLATED CHICKEN EYE TEST – THE MOST COMMON HISTOPATHOLOGICAL CHANGES

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Introduction: The isolated chicken eye (ICE) test is an alternative method accepted by the OECD for identification of severe irritants. Histopathological examination of the corneas is not required, however OECD guidelines (no. 438) encourages this conduct.

Materials and Methods: After the routinely performed ICE tests, the total number of 387 chicken eyeballs were collected, fixed in a 4% neutral buffered formaldehyde solution, and then trimmed in the horizontal plane containing cornea and adjacent sclera, lens, and optic nerve. So prepared slices were dehydrated, embedded in paraffin, and stained with HE.

Results: Examined samples have mostly shown changes at the level of the anterior corneal epithelium, without major lesions in the corneal stroma or posterior corneal epithelium. Dominant lesions in the corneas revealed by microscopic examination were exfoliation and wane of the anterior corneal epithelium. The cell swelling and cell vacuolation of the anterior corneal epithelium, and the dissection, detachment or lack of anterior corneal epithelium was also observed. Although most changes were limited to the anterior corneal epithelium, dissection of the corneal stroma was also frequently visible. However, the latter were not associated with the test items.

Conclusions: Although the histopathology of the cornea is an additional test, our research shows that in 30% of cases it is a very useful tool to clarify borderline effects that help to quantify test effects.

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Poster Presentations ESVP/ECVP

P079 PERISPLENIC FIBROINFLAMMATORY ATYPICAL POLYPS IN A KITTEN

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Introduction: Nodular masses of the spleen often represent a frustrating issue both for clinicians and for pathologists. Frequently a neoplastic process is identifiable, and often times it is a sarcoma with a poor prognosis. Many times, however, hemorrhagic haematomas in the spleen lead to splenectomy with or without emergency surgery after rupture, without possible identification of primary/metastatic tumours as their cause. Investigating nature and pathogenesis of splenic masses would therefore add knowledge and potentiate clinical approach to this kind of lesions.

Materials and Methods: Here we describe clinical, gross, histological, and follow up data of an unusual case of perisplenic nodules in a 7-month-old, male, spayed kitten, presented with weakness and inappetence. Complete splenectomy and histology were performed.

Results: At gross examination multiple exophytic, irregularly-sized (1-5cm) nodules were evidenced on the spleen. These nodules at histology were characterised by a fibrovascular proliferation arising from the splenic capsule. Immature fibroblasts and endothelial cells were admixed with a diffuse eosinophilic infiltration and occasional mononuclear cells. Cellularity was variable with very oedematous areas and loose extracellular matrix. Atypia was moderate with occasional mitoses. Follow up evidenced remission of symptoms but further development of new nodules in the site of surgery after 2 months.

Discussion (and/or Conclusions): On the bases of the results the lesion was considered consistent with an atypical fibrovascular proliferation with an eosinophilic component. To our knowledge no similar lesions have been described in veterinary medicine. The most likely differential diagnoses include inflammatory pseudotumour/fibro-inflammatory polyp and a low-grade sarcoma.

Notes:

P080 TLR-9 AND PARASITE DNA IN THE CHOROID PLEXUS MAY IMPAIR THE BLOOD-CSF BARRIER OF DOGS INFECTED WITH *LEISHMANIA INFANTUM*

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Introduction: The central nervous system (CNS) undergoes inflammatory alterations during peripheral infection by the protozoan *Leishmania infantum*, including lymphocyte infiltration and glial activation. The aim of this study was to detect the gene expression of TLR-9, the parasite DNA in the CNS of dogs with visceral leishmaniasis (VL) and to assess the integrity of the blood-cerebrospinal fluid (CSF) barrier.

Materials and Methods: Brain fragments and CSF samples of 15 dogs with VL and four uninfected dogs were collected. TLR-9 gene expression was assessed by Taqman RT-qPCR, the parasite DNA was detected by qPCR and by *in situ* hybridisation, and the integrity of the blood-CSF barrier was assessed by the Albumin quota.

Results: TLR-9 gene expression was up-regulated in the choroid plexi of the infected dogs but not in the whole brain tissue. There was noticed high amount of parasite DNA in the brain and we detected parasite DNA deposition around blood vessels of the choroid plexi of 78.6% of the dogs by in situ hybridisation. By the albumin quota, we detected alteration in 20% of the dogs whereas 13.3% were near the cut-off value. Further, 60% presented high anti-*Leishmania* antibody titers in the CSF.

Discussion (and/or Conclusions): The exclusive expression of TLR-9 in the choroid plexus and the deposition of parasite DNA around the blood vessels may indicate that there is in course an immune activation in this structure, allowing the production of cytokines, such as IL-1β, IL-6 and TNF-α, which are able to disrupt the blood-brain-barrier.

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Poster Presentations ESVP/ECVP

P081 SPINAL CORD LESIONS IN A CAT INFECTED WITH *GURLTIA PARALYSANS*

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Introduction: *Gurltia paralysans* is a rare metastrongyloid nematode found mainly in the leptomeningeal veins and parenchyma of the spinal cord of domestic and wild cats in Chile, Argentina, Brazil, Uruguay and Colombia. Here, subsequent lesions of the lumbar spinal cord in a 3 year old male domestic cat originally from Punucapa (Los Rios region, Chile) with chronic paraparesis are described.

Materials and Methods: Nervous tissue was stained with haematoxylin-eosin, Luxol fast blue, van Gieson and congored and activation of glial and endothelial cells and immune cell infiltration was visualised by immunohistological markers (GFAP, CNPase, Factor VIII, MAC 387, CD3, CD45R).

Results: Histologically, in the meningeal veins of the spinal cord intravascular nematode larvae and pre-adult stages, subsequent congestion, thrombosis and thickening of subarachnoidal vessels were found. Spinal cord parenchyma was affected by multiple haemorrhages and showed extensive foci of malacia with gitter cells and adjacent gliosis. Primarily lymphocytes intermingled with fewer macrophages infiltrated the meninges forming a perivascular pattern.

Discussion: Neurological signs can be attributed to the severe chronic lesions in the spinal cord subsequent to ischaemia due to parasitic vascular injury.

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P082 SYSTEMIC MYCOSIS CAUSED BY *CRYPTOCOCCUS NEOFORMANS* IN A CAT

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Introduction: Systemic mycotic infection was diagnosed in a 1-year-old female domestic shorthair cat with poor body condition, swelling in the dorsal nasal region, exophthalmic right eye, swelling and ulceration of the soft palate, enlarged mandibular, lateral and medial retropharyngeal lymph nodes and purulent nasal discharge.

Materials and methods: After the macroscopic examination tissue samples were collected for cytological, histopathological and microbiological examination. Air dried cytological smears were stained with Diff-quick stain. Tissue samples for histopathological examination were fixed in 10% buffered formalin, dehydrated, embedded in paraffin blocks, and 5 µm thick sections were stained with haematoxylin and eosin (H&E), Periodic acid Schiff (PAS) and Grocott stain for the detection of infection agents in tissues. For the isolation of the microorganisms conventional microbiological methods were used, and the Fungifast® system (Elitech Group) was used for the identification of the yeast.

Results: Macroscopically, a whitish tumour-like mass occupied dermis of the dorsal nasal region and most of the nasal cavity and maxillary and frontal sinus. Purulent nasal discharge, as well as swelling and ulceration were present on the soft palate. Mandibular, lateral and medial retropharyngeal lymph nodes were enlarged. Microscopically the lesions contained abundant organisms surrounded by confluent unstained capsules which create a pale, foamy ("soap bubble") low-power appearance with scant inflammation. The yeast was PAS and Grocott positive. Cytologically examination revealed spherical organism with thick clear-staining mucoid capsule.

Conclusion: This report describes fatal systemic *Cryptococcus neoformans* infection in a cat that was confirmed by culture.

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Poster Presentations ESVP/ECVP

P083 CONCURRENT GRANULOMATOUS NEPHRITIS AND COLITIS ASSOCIATED WITH INVASIVE *ESCHERICHIA COLI* IN A BOXER DOG

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Introduction: Granulomatous colitis is a condition associated with chronic mucoid, haemorrhagic diarrhoea in young Boxer dogs and French Bulldogs.

Materials and Methods: A 7 month-old female Boxer was presented with recurrent cystitis and azotaemia. Urine culture grew a multi-drug resistant *Escherichia coli*. Treatment with cefovecin resulted in improvement of the cystitis and stabilisation of the azotaemia for 6 months. She then developed haemorrhagic colitis from which faecal beta-haemolytic *E. coli* was isolated. Despite treatment, the azotaemia and colitis progressed and the dog was eventually euthanised 16 months after initial presentation.

Results: Post mortem examination revealed irregular, tan, firm, mass-like enlargement of both kidneys at their cranial poles. Colonic mucosa was thickened with multifocal erosions. Histopathology revealed large numbers of PAS-positive macrophages effacing the colonic mucosa and submucosa as well as multifocal areas of the renal cortices and medullae. Fluorescence *in situ* hybridisation revealed multifocal intrahistiocytic clusters of *E.coli* in the colon and kidney.

Discussion (and/or Conclusions): This is the first description of concurrent granulomatous nephritis and colitis associated with invasive *Escherichia coli*. These results further support the hypothesis of an underlying defect in host immune response against *E.coli* as the basis for this syndrome, which is not restricted or specific to the colonic mucosa.

P084 THE HISTOPATHOLOGICAL MANIFESTATION OF CHRONIC HEART BORRELIOSIS IN DOGS

Notes:

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Introduction: Borreliosis is an infectious disease caused by *Borrelia burgdorferi* sensu lato. Clinical signs of cardiac borreliosis are nonspecific and include rhythm disturbances and heart failure symptoms. The disease in dogs is rarely confirmed because of difficulties in presenting spirochetes in standard bacteriological and histological staining.

Materials and Methods: The study was carried out on a group of 6 dogs showing nonspecific signs of heart failure and positive antibody titer against *Borrelia sp*. A post-mortem histologic examination performed on tissue samples from both atria, both ventricular walls and interventricular septum included HE and immunohistochemical staining towards *Borrelia burgdorferi* sensu lato (polyclonal antibody, AbD Serotec, UK, serial no 1439-9406).

Results: Signs of diffuse lymphocytic infiltrates combined with symptoms of cardiomyocytes degeneration and the presence of *Borrelia burgdorferi* sensu lato spores were stated in the examined slides.

Discussion: The inflammatory infiltrates combined with acute cardiac borreliosis most often consist of intense active transmural interstitial lymphocytes. In chronic forms it changes towards diffuse subendocardial lymphocytic infiltrates. The cardiomyocytes degeneration may be a result of direct influence of spirochetes or a consequence of microorganisms' toxins or autoimmunological reaction. Presenting *Borrelia burgdorferi* sensu lato in heart specimens requires special immunohistochemical staining. To best of our knowledge this is the first study describing the presence of spore forms of those spirochetes in dog heart samples.

Poster Presentations ESVP/ECVP

P085 CASE OF AN OCULAR PROTOTHECOSIS IN A YOUNG DOG

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Introduction: Protothecosis is a rare opportunistic algal disease in dogs. Often disseminated visceral and/ or cutaneous infections occur. Ocular manifestation is described in association with systemic manifestation. In this particular case the dog presented only a diseased eye with no signs of systemic or cutaneous infection.

Materials and Methods: A 11-month-old, intact male, Labrador Retriever dog presented clinically an effusion-caused, retinal detachment and an elevated intraocular pressure in his right eye. The dog was in good general condition and the eye was surgically removed because of the glaucoma. The bulbus was processed for histopathological examination, and samples were submitted for PCR and sequence analysis.

Results: Histopathology revealed a subretinal mass composed of thin-walled, oval structures which measured up to 50 x 30 µm. Most of them had no internal structures and very rarely endospores could be demonstrated ("cut pie" appearance). Between the organisms and diffusely in the uvea posterior numerous macrophages and plasma cells were seen. Sequence comparison revealed a 94% congruence with *Prototheca zopfii*.

Discussion: A diagnosis of an ocular protothecosis was made based on histological features, PCR and sequence analysis. Other inflammatory or neoplastic lesions were ruled-out by pathohistological examination. 7 months before enucleation the dog had a short episode of diarrhoea but recent examination of faeces and urine could not reveal algae. It was not possible to prove if the intestine was the portal of entry for the algal infection.

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P086 OCULAR AND PODODERMAL MYCOSIS WITH *ASPERGILLUS NIDULANS* IN A DOG

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Introduction: A 7-year-old, female German Spaniel dog, previously long-term treated with prednisolone, presented with loss of vision with severe, unilateral panophthalmitis of the left eye. Clinically, massive uveitis with posterior synechiae, retinal detachment, and progressive glaucoma were diagnosed and the eye was enucleated 12 days after onset of clinical signs. Four days later, the dog developed progressive, therapy resistant pododermatitis.

Materials and Methods: Samples were routinely processed for histology and stained with haematoxylin & eosin and the periodic acid-Schiff (PAS) reaction. Additionally, DNA was isolated from formalin fixed, paraffin-embedded samples and tested by PCR using fungal *ITS-1* gene specific primers, followed by amplicon sequencing.

Results: Histology revealed chronic-active, granulomatous panophthalmitis and pododermatitis with intralesional fungal hyphae suggestive of *Aspergillus* spp. PCR confirmed the fungus belonging to the genus Aspergillus and comparison of sequencing results using Basic Local Alignment Search Tool (BLAST) revealed 100% DNA sequence identity with *Aspergillus nidulans* in both samples.

Discussion: The absence of other ocular lesions and the primary intraocular manifestation in terms of panuveitis suggests an underlying systemic haematogenous mycotic spread, probably as a consequence of immune suppression due to the long-term administration of prednisolone. Although other *Aspergillus* spp. are commonly seen in local and systemic mycoses in various species, *Aspergillus nidulans* as an unusual pathogen has not been described as cause of panophthalmitis or pododermatitis before.

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Poster Presentations ESVP/ECVP

P087 FATAL MENINGOENCEPHALITIS CAUSED BY *HALICEPHALOBUS GINGIVALIS* IN A STALLION IN PIEDMONT, ITALY

Notes:

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Introduction: *Halicephalobus gingivalis* is a free-living nematode belonging to the order Rhabditida. Commonly it is found in association with soil, manure and decaying organic matter and affects horse, zebra and humans. The aetiopathogenesis is not known. A definitive diagnosis is possible in vivo by biopsy in the accessible nodular lesions or post-mortem by histology.

Materials and Methods: A thirteen-year-old Koninklijk Warmbloed Paard Nederland stallion, residing in a farm in Turin province, was submitted to clinical examination after a 2-day history of a severe and rapidly progressive neurological disorder. The stallion, suspected of West Nile virus (WNV) infection, was euthanised and necropsy was performed. Head and blood samples were sent to the Neuropathology Laboratory of the Istituto Zooprofilattico Sperimentale of Turin for investigations.

Results: WNV blood testing was negative. Neuropathological findings were consistent with a verminous meningoencephalitis predominantly affecting the basal ganglia and thalamus. A large numbers of macrophages, lymphocytes, eosinophils and multinucleated giant cells formed perivascular cuffs surrounded by malacic areas infiltrated with many gitter cells and with evident axonal spheroids. Several larvae and eggs of *H. gingivalis* were found. Molecular and phylogenetic analyses based on the nuclear large subunit ribosomal RNA (LSU rDNA) gene are in progress.

Discussion and Conclusions: The horse was native to The Netherlands and lived with other healthy horses in Italy from 2004. Probably the horse was infected after its housing in Italy. Phylogeny could help to understanding the geographic origin of the parasite and to estimate the risk for humans.

P088 IMMUNOHISTOCHEMICAL CHARACTERISATION OF NEURODEGENERATION AND ASTROCYTOSIS OF LESIONS IN CENTRAL NERVOUS SYSTEM OF CATTLE WITH RABIES

Notes:

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Introduction: Rabies is a neurological disease caused by a neurotropic virus of the *Rhabdoviridae* family, resulting in acute, progressive, fatal encephalitis. It affects all mammals, being a major zoonosis worldwide. In April 2014, three outbreaks of rabies were described affecting cattle in Uruguay near the border with Brazil, where it is endemic. Histologically, rabies causes viral encephalitis with intracytoplasmic inclusion bodies (Negri bodies), but these may be absent. Definitive diagnosis is made with FAT or immunohistochemistry (IHC). The aim of this study was to detect viral antigen in cattle with rabies, characterising lesions in the central nervous system (CNS) by IHC, using antibodies against rabies, GFAP and calbindin 28kd (Cb28kd).

Materials and Methods: Three CNS of crossbreed bovines with histopathologic diagnosis of rabies, and 1 normal cow as control were used. Samples were taken from cerebral cortex, cerebellum, hippocampus and pons. IHC were carried out using the following primary antibodies: anti-rabies (polyclonal, Chemicon, recommended for FAT), 1:1000 dilution; anti-GFAP (monoclonal, Biocare), and pre-diluted anti-Cb28kd (monoclonal, Novocastra), 1:500 dilution.

Results: Rabies antigen was detected in different neuronal types in all cases diagnosed previously. In these cases, neurodegeneration was accompanied by reduction of Cb28kd immunoreaction in mainly in GABAergic neurons, reactive astrocytosis was also identified by GFAP in these cases.

Conclusions: We detected the presence of rabies viral antigens in different regions of CNS, showing a progressive loss of Cb28kd immunoreactive neurons (particularly in cerebellar Purkinje cells), accompanied by reactive astrogliosis.

Poster Presentations ESVP/ECVP

P089 NEUROPATHOLOGY OF MENINGOENCEPHALITIS IN SEPTIC PIEDMONTESE CALVES: A PRELIMINARY STUDY

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Introduction: Septic calves can develop meningoencephalitis (ME). Here we describe the brain lesions and bacteriology results in septic calves.

Materials and Methods: Twenty-seven, 1 to 15-day-old, septic Piedmontese calves were examined grossly and histopathologically. Immunohistochemistry (IHC) was performed to evaluate the presence of *Bovine Viral Diarrhoea Virus, Bovine Herpesvirus 1, Listeria monocytogenes, Chlamydophila* spp, *Flavivirus, Neospora* spp., and *Morbillivirus*. Bacteriology was performed on brain, spleen, liver and femoral bone marrow.

Results: Brain lesions were detected in 21 calves. Lesions included meningoencephalitis (13.62%) and meningitis (8.38%) with no neuroparenchymal involvement. Meningeal infiltrate was suppurative in 12 cases (57.2%) and non suppurative in 9 animals (42.8%). *Escherichia coli* was isolated in 20 animals (74%, 8 with neutrophils and 7 with no neutrophils).

Discussion (and/or Conclusions): Brain lesions in neonatal calves affected by sepsis differ in type and distribution of inflammatory cell infiltrate. Chronicity of infection, antibiotic treatment, endotoxin and inflammatory cytokines are all variable possibly able to influence the inflammatory response. Further studies should be done to clarify the pathogenesis of the lesions.

Notes:

P090 RELATION OF TISSUE CYST NUMBER, HISTOPATHOLOGY SCORE AND SYSTEMIC ACUTE PHASE PROTEINS IN EXPERIMENTALLY *TOXOPLASMA GONDII* (ME49 STRAIN) INFECTED MICE

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Introduction: Toxoplasmosis is a disease caused by the protozoan *Toxoplasma gondii*, which occurs worldwide in mammals and birds. The brain is the primary target organ because *T. gondii* is a ubiquitous intracellular parasite that most frequently causes life-threatening encephalitis in immunocompromised patients. Relation of tissue cyst number, histopathology score and acute phase proteins were investigated.

Materials and Methods: In this study, 36 mice were infected with Me49 strain of *T. gondii*. The control group had 6 healthy mice. After inoculation with *T. gondii*, 6 mice were euthanised after collection of blood samples at days 10, 15, 20, 30, 45, or 60. Haemopexin, haptoglobulin, macroglobulin, SAA (Serum amyloid A) and clusterin levels were determined by ELISA. Brain tissues were investigated histopathologically and lesions were scored. Average cyst numbers were determined by counting three samples (25 µl each) of each brain homogenate under light microscopy.

Results: Inflammatory reaction was observed on day 10 p.i. The lesions were characterised by perivascular mononuclear cell infiltration, focal mononuclear cell infiltration in the meninges, and glial proliferation. Tissue cysts were observed in all *T. gondii*-infected groups. The highest lesion score was on day 60 p.i. and the highest tissue cyst number was on day 30 p.i. Serum levels of haemopexin, haptoglobulin, macroglobulin, SAA and clusterin were significantly higher than in the control group on days 10-20, 10, 10-30, 10,10-45 p.i., respectively.

Discussion: High levels of acute phase proteins in mice infected with *T. gondii* on certain days appear to reflect a relationship between brain lesions and tissue cysts.

Notes:

Poster Presentations ESVP/ECVP

P091 A NOVEL MOUSE MODEL FOR THE STUDY OF URBAN-RIFKIN-DAVIS SYNDROME (URDS)

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Introduction: Urban-Rifkin-Davis-syndrome (URDS) has been recently described as a novel connective tissue disorder caused by mutations in latent TGF-β binding protein 4 (LTBP4). Most URDS patients present with high mortality due to respiratory failure in infancy or early childhood. To gain insight into the molecular function of LTBP4 for tissue integrity and homeostasis we generated a complete LTBP4 knock-out mouse line by ablating both the short and the long transcript variant by a gene-trap approach (*Ltbp4S/L*$^{-/-}$).

Methods: *Ltbp4S*$^{-/-}$ and *Ltbp4S/L*$^{-/-}$ mice were generated by a gene trap approach and phenotypically and biochemically analysed in comparison. Biochemical and molecular analyses using q PCR, Biacore, Western blots provided further insight into the underlying disease causing molecular mechanisms initiated by complete LTBP-4 ablation. Morphological analysis (histopathology, ultrastructure, immunohistochemistry) identified a distorted elastic fiber and extracellular matrix network.

Results and Conclusions: It could be demonstrated that *Ltbp4S/L*$^{-/-}$ mice fully resemble human URDS as evidenced by the following findings: *Ltbp4S/L*$^{-/-}$ mice died within two weeks after birth, showed a decrease in body weight and growth reduction, developed pulmonary emphysema as well as right ventricular hypertrophy, and showed a severely distorted elastic fiber network . We identified Fibulin-4 as a new interaction partner for both LTBP-4 isoforms and further demonstrated that the interaction with LTBP-4L was required for the linear deposition of fibulin-4. This model will contribute to the current understanding of how the elastic fiber network is formed but also provide future opportunities to study URDS in an animal model.

Notes:

P092 MULTIFOCAL FIBROSING MYOCARDITIS IN A DOG

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Introduction: Heart failure associated with chronic myocardial fibrotic lesions is not common in the dog, and it can be related to chronic infectious myocarditis and infarcts. Immunomediated myocarditis is described in humans. Most commonly, immunomediated disorders in the dog affect the skin.

Materials and Methods: Here we present an unusual case of a dog, 7-years old, dachshund, male, with multifocal cutaneous lesions, that died of heart failure with liver involvement after prolonged cortisone treatment.

Results: Necropsy showed cutaneous, hepatic and heart lesions. Hepatomegaly was associated with multifocal nodular whitish lesions and accentuated lobular pattern. The skin showed multifocal alopecia and plaque-like oval lesions. Grossly the heart manifested mild multifocal ventricular whitish discolouration. Histology evidenced unusual atrophic and fibrotic lesions in the heart with a focally extensive infiltrate of mononuclear lymphoid cells. In the liver a pattern of porto-portal severe degeneration and cholestasis was observed with lack of the smaller bile ducts. In the skin a chronic hyperplastic plaque-like dermatitis was present with infiltration of mononuclear lymphoid cells. Immunohistochemistry revealed a mild positive reaction to CD3 and CD5 in the skin and heart infiltrate with rare CD79+/CD20+ cells. All major infectious diseases were excluded.

Discussion (and/or Conclusions): On the basis of these results, a chronic hepatic disorder with associated cutaneous involvement and chronic heart damage was diagnosed. An immune-mediated disorder as primary cause is discussed with review of the literature. The lymphoid infiltrate in the heart and skin show signs of atypia and a progression to lymphoma is also considered.

Notes:

Poster Presentations ESVP/ECVP

P093 PREDUCTAL AORTIC COARCTATION AND PATENT DUCTUS ARTERIOSUS IN A 5-MONTH-OLD KITTEN

Notes:

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Introduction: Congenital malformations of the aorta, leading to heart failure are a well described phenomenon in infants, but rarely seen in the domestic cat (*Felis catus*).

Materials and Methods: A 5-month old, male kitten was presented with increasing symptoms of inspiratory and expiratory dyspnoea over a 7 day period. Due to increasing cyanosis, lung congestion, abdominal transudate and the lack of response on treatment, the animal was euthanised. Given its age and symptoms a post mortal examination was conducted to reveal any anomaly regarding the cardiovascular system, and if present whether congenital and potentially hereditary.

Results: The abdominal and thoracic cavities were filled with transudate and showing an enlarged spleen and severely congested lungs. Close macroscopic examination of the heart exposed a malformation of the aorta, known as preductal coartaction, and a persistent ductus arteriosus.

Discussion (and/or Conclusions): Aortic coarctation, a congenital narrowing of the aorta, comes in two distinct types. Juxtaductal coarctation is a localised narrowing of the aortic wall due to a discrete, shelf-like thickening and is generally found at the junction of the aortic isthmus, ductus arteriosus and descending aorta. Preductal coarctation is a diffuse narrowing, caused by tubular hypoplasia of the aorta. In felines, this congenital anomaly has only been described once in a Sumatran tiger cub. This is the first case in a domesticated cat. Review of the literature indicates an increase in the diagnosis of congenital heart diseases in small animals. More specifically in cats, common congenital defects are atrioventricular dysplasia and ventricular septal defects.

P094 A CASE OF FELINE RENAL OXALOSIS WITH VASCULAR INVOLVEMENT

Notes:

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Introduction: Two types of oxalosis occur in cats, an acquired and a heritable disorder (primary hyperoxaluria – PH). PH is a disease of young cats, homologue to primary hyperoxaluria type 2 in humans (PH2) which results from mutations in the glyoxylate reductase (GRHPR) gene.

Materials and Methods: A 7 month old European shorthair female cat with a one week history of anorexia and neurologic signs confined to the right hind limb (plantigrade stance) was examined. The affected limb was with proprioceptive deficit, paresis, pain, poikilothermia, no arterial pulse and cyanotic digital pads. The cat died within 12 hours of admission and was submitted for post-mortem examination. Biochemical, histopathological and histochemical exams were performed.

Results: Biochemical profile revealed severely increased levels of urea, phosphorus and AST, and normal iPTH levels. Gross findings included intercostal subpleural mineralisation, irregular thickening of the wall of major arteries, pale right hind limb muscles and thrombosis of the right femoral artery. Microscopically tubular nephrosis with intralesional calcium oxalate crystals and prominent myocardial and vascular (mainly arterial) metastatic mineralisation were observed.

Discussion: Considering the age, history and lesions a presumptive diagnosis of feline PH with widespread vascular involvement was given. Vascular lesions have not been previously described in cats with PH.

Poster Presentations ESVP/ECVP

P095 SEVERE CONGENITAL PLEUROPERITONEAL DIAPHRAGMATIC HERNIA AND PERICARDIAL APLASIA IN A FRIESIAN STILLBORN FOAL

Notes:

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Introduction: Congenital diaphragmatic hernia (CDH) and pericardial aplasia are largely idiopathic and uncommon lesions in animals.

Materials and Methods: A full term female Friesian stillborn foal was presented for necropsy examination. The pedigree was also made available for a complete analysis.

Results: Grossly the peritoneal cavity contained approximately 500 ml of clear liquid. The small intestine, caecum, ascending colon, spleen and the intermediate-left liver lobe were displaced in the thoracic cavity through a diaphragmatic defect measuring 20/15 cm. The diaphragmatic defect involved the left-dorsal part of the diaphragm tendinous center. The complete absence of the anterior and left pericardium and hypoplasia of the left lung was observed, associated also with abdominal organ displacement. The displaced lobe of the liver was severely enlarged and showing marked diffuse fibrosis.

Discussion (and/or Conclusions): Congenital diaphragmatic hernias are uncommon lesions in horse, however several studies reported different types of hernia including typical pleuroperitoneal diaphragmatic hernia, hiatal hernia, peritoneopericardial diverticulum and retrosternal diaphragmatic hernia. Herein, this is the first report of a congenital diaphragmatic hernia in Friesians and the first mention of a CDH and pericardial aplasia in foal. In humans there are a number of studies which suggest that genetic factors are involved in development of CDH but in our case this fact could not be confirmed. The pedigree analysis showed no similar phenotype resulting from the same genitors.

P096 INCREASED MORTALITY IN CAPTIVE ROCK HYRAXES (*PROCAVIA CAPENSIS*) AT RIGA NATIONAL ZOO (LATVIA) ASSOCIATED WITH SYSTEMIC APICOMPLEXAN INFECTION, HIGHLY SUSPICIOUS OF *TOXOPLASMA GONDII*, AND HAEMOSIDEROSIS

Notes:

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Introduction: Apicomplexa are protozoal parasites infecting most genera of warm-blooded animals. A typical feature for this phyllum is the formation of sporocysts which produce infectious sporozoites. The diseases caused by this group of organisms include among others sarcocystosis, neosporosis and toxoplasmosis. In the period from October 2012 till November 2013 in Riga National Zoo of Latvia 14 rock hyraxes showed signs of generalised weakness followed by death. The outbreak started after animals were moved to an enclosure located next to the wild cats.

Materials and methods: Formalin fixed, paraffin embedded tissue samples from various organs (heart, liver, lung, kidney, uterus, spleen, thyroid gland, lymph nodes, bone marrow, intestine, brain) from all animals were histologically examined using H&E, Periodic Acid Schiff, Prussian Blue staining, immunohistochemistry (IHC) and were tested by PCR

Results: Histological examination revealed parasitic sporocysts in the heart, liver and uterus (9/14), coagulation necrosis with mononuclear (lymphoplasmacytic, histiocytic) infiltrates (14/14) in virtually all organs, and haemosiderosis in the liver (10/14). Intralesional parasites histologically compatible with genus Apicomplexa were positive for *Toxoplasma gondii* specific antibodies while PCR product sequencing failed to specify the species.

Discussion and conclusions: This case report shows that hyraxes seem to be susceptible to infections with apicomplexan protozoa. Immunohistochemical analysis indicates the involvement of *Toxoplasma* spp. in the disease. Another factor that could play a role in the pathogenesis of the disease may be the haemosiderosis in most animals, which is known to suppress the immunity of affected animals, causing an increased susceptibility for other diseases.

Poster Presentations ESVP/ECVP

P097 HELICOBACTER INFECTION IN CHEETAHS (*ACINONYX JUBATUS*): FROM CHRONIC LYMPHOCYTIC GASTRITIS TO REFLUX OESOPHAGITIS WITH INTESTINAL METAPLASIA

Notes:

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Introduction: Worldwide, the majority of captive cheetahs developed a progressive *Helicobacter*-associated chronic gastritis causing vomiting, weight loss, and failure to thrive. We described two cases of Helicobacter-like organisms (GHLOs) associated gastritis with gastro-oesophageal reflux in cheetahs, with a particular condition of intestinal-type columnar metaplasia of the pre-cardial oesophageal mucosa.

Materials and Methods: A chronic diffuse gastritis and reflux oesophagitis from cardial incompetence was endoscopically diagnosed in two cheetahs, a 7 years old male and a 4 years-old female, with a long history of recurrent vomiting and dysphagia, maintained in an Italian Zoo. For the histological analysis, biopsies were processed routinely, and sections stained with H&E, Alcian-PAS (pH 2.5) and Giemsa. Inflammatory infiltrates and GHLOs organism were characterised also by immunohistochemistry.

Results: A GHLOs-related gastritis, with florid CD3+/CD4+/CD25-/Foxp3- T cells and CD79a+/CD21+ B cells infiltrates, and with CD3+/CD8+ T cells within the glandular epithelium was diagnosed in both cheetahs. Chronic oesophagitis was also observed in both animals and, in male oesophageal biopsies, an intestinal metaplasia with columnar, and goblet cells rich epithelium, heavily GHLOs-colonised, was also observed.

Discussion: In captive cheetahs, the most common causative agent of chronic gastritis is represented by *Helicobacter* spp. infection. Although there are many descriptions of this GHLOs-related gastritis in cheetah, there are no reports on the potential impacts that this chronic condition and recurrent vomiting can have on the oesophageal mucosa. We describe two cases of GERD in cheetahs, differing for degree of severity, with a case of intestinal-type columnar metaplasia of the pre-cardial esophageal mucosa, associated with GHLOs colonisation. This condition, known also as *Barrett's* oesophagus, is one of the possible complications of reflux oesophagitis in man, rarely reported in animals (Gibson, 2010; Gualtieri, 2006) and, until now, undescribed in cheetahs.

P098 DETECTION OF A *PNEUMONIA VIRUS OF MICE* (PVM) IN AN AFRICAN HEDGEHOG (*ATERELIX ARBIVENTRIS*) WITH SUSPECTED WOBBLY HEDGEHOG SYNDROME (WHS)

Notes:

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Introduction: Wobbly hedgehog syndrome (WHS) is an idiopathic progressive paralysis of African hedgehogs.

Materials and Methods: A 198-days-old female pet African hedgehog was presented with sudden onset of astasia and abasia. The practitioner suspected WHS. The clinical symptoms deteriorated and the animal died 51 days after presentation. The animal was subjected to pathological examination, including immunohistochemistry with mouse anti-pneumonia virus of mice (PVM) (strain 15) antisera (kindly supplied by Dr. H. Rosenberg). Total RNA was extracted from formalin-fixed, paraffin-embedded hedgehog brain tissue. Libraries for deep sequencing were constructed and sequence data analysis was performed.

Results: A PVM from an African hedgehog with suspected WHS was detected and genetically characterised. The affected hedgehog had a nonsuppurative encephalitis with vacuolisation of the white matter, and the brain samples yielded RNA reads highly homogeneous to PVM (strain 15) (96.5% of full genomic sequence homology by analysis of next generation sequencing). PVM antigen was also detected in the brain and the lungs immunohistochemically.

Discussion (and/or Conclusions): A PVM was strongly suggested as a causative agent of encephalitis of a hedgehog with suspected WHS. This is a first report of PVM infection in hedgehogs.

Poster Presentations ESVP/ECVP

P099 RETROSPECTIVE STUDY OF AETIOLOGIC AGENTS ASSOCIATED WITH NONSUPPURATIVE MENINGOENCEPHALITIS IN STRANDED CETACEANS IN THE CANARY ISLANDS

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Introduction: *Dolphin morbillivirus* is the main agent described in cases of nonsuppurative (n-s) meningoencephalitis in striped dolphins during the last two epizootics in the Mediterranean Sea. Other causative agents of this pathology in marine mammals include herpesviruses; *Toxoplasma gondii*; and *Brucella* spp. Several cases of n-s meningoencephalitis have been described in cetaceans stranded along the coasts of the Canary Islands, although the associated aetiology remains undetermined.

Materials and Methods: Nineteen cases of encephalitis in free-ranging cetaceans from three different species (striped, common, and rough-toothed dolphins) were studied retrospectively. Histological examination of the brains revealed variable degrees of n-s meningoencephalitis, characterised predominantly by perivascular lymphohistiocytic infiltrates. Polymerase chain reaction (PCR) assay was used on brain and other available tissues to detect the presence of the aforementioned agents. In addition, immunohistochemical (IHC) staining was performed on selected tissues for the occurrence of morbilliviral antigen.

Results: Six animals (5 striped dolphins and 1 common dolphin) showed IHC and/or molecular evidence of morbilliviral antigen and/or genome in brain tissue. Herpesviral DNA was detected in brain tissue from two striped dolphins. In 11 animals there was no positive reaction against the tested agents.

Discussion (and/or Conclusions): The information presented herein increases the number of confirmed morbillivirus positive cases within the Canarian Archipelago. Regarding herpesvirus, the phylogenetic analysis provides valuable information about a possible pathogenic branch of cetacean alphaherpesviruses that might be responsible for some fatal cases worldwide. Further studies are needed to investigate the involvement of other aetiological agents causing meningoencephalitis in cetaceans.

Notes:

P100 HISTOPATHOLOGICAL FINDINGS IN CETACEANS STRANDED ON THE SPANISH MEDITERRANEAN COAST BETWEEN 2011 AND 2014

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Introduction: A histopathological study was carried out on 11 cetaceans stranded on the southeastern Spanish Mediterranean Coast, between 2011 and 2014. The purpose of this study was to contribute to the surveillance of the health status of the free-ranging cetacean population.

Materials and Methods: Formalin-fixed tissue samples were submitted to the Pathology Service of the Complutense University Veterinary Hospital, from 8 striped dolphins (*Stenella coeruleoalba*), 2 Risso's dolphins (*Grampus griseus*), and 1 Cuvier´s beaked whale (*Ziphius cavirostris*), for examination.

Results: Mortality was due to trauma in 46% of the animals, infectious disease in 36% and unknown in 18% of the cases. The most frequent findings were chronic interstitial nephritis (55%), granulomatous pneumonia with intralesional metastrongyles (54%), lymphoid depletion (45%), meningoencephalitis (27%), chronic active hepatitis (18%), and granulomatous colitis with intralesional cestodes (18%). Incidental findings were an adrenal vascular hamartoma, a granulomatous lymphadenitis and orchitis.

Viral pneumonia and/or meningoencephalitis consistent with *Dolphin morbillivirus* (DMV) infection was noted in one of the striped dolphins. Two striped dolphins had mild chronic encephalitis suggestive of a prior or subclinical viral infection.

Discussion (and/or Conclusions): Infectious disease of some sort was found in all of the animals and not necessarily related to cause of death. Mortality was mainly due to trauma, followed by infectious disease (septicemia, viral, etc.). A generalised lymphoid depletion, suggestive of immune compromise, may favor certain infections. Similar studies in other parts of the Mediterranean have hypothesised an association between lymphoid depletion and environmental contaminants. Other possible causes remain to be elucidated.

Notes:

Poster Presentations ESVP/ECVP

P101 FOOT LESIONS IN FARMED MINK (*NEOVISON VISON*)

Notes:

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Introduction: The occurrence of calluses on the feet of mink has been mentioned only briefly in the literature; however, examination of feet from Danish farmed mink has revealed a large proportion of callus-like lesions in the plantar metatarsal area. The aim of this study was to evaluate pathologic, histopathologic and epidemiologic features of foot lesions in farmed mink.

Materials and Methods: The study included 1163 mink from four Danish mink farms. The selection of farms was based on history of foot lesions on all four farms. All feet including the carpus and tarsus were examined and lesions described macroscopically. For histologic characterisation, 22 tissue samples from a representative spectrum of feet with and without lesions were examined. Macroscopic and microscopic appearance was compared to that of 47 free-ranging Danish mink.

Results: Feet were grouped according to gross inspection as: no lesions (55.1%), hair loss (7.1%), hyperkeratosis (35.8%) or crusting (5.3%). Lesions were predominantly located on the plantar surface of the metatarsal area (98.1%). Histologically, lesions included variable degrees of orthokeratotic hyperkeratosis and granulomatous to pyogranulomatous dermal inflammation with trichogranulomas as a dominating feature in all farmed and several free-ranging mink.

Discussion and Conclusion: The gross and microscopic lesions resemble physically induced changes as described in other species which develop as a response to repeated friction or pressure to the plantar and palmar skin area.

P102 GENERALISED GRANULOMATOUS DISEASE IN A HUMBOLDT PENGUIN (*SPHENISCUS HUMBOLDTI*)

Notes:

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Introduction: Penguins are avian species with special husbandry needs and the frequent captivity pathology includes mycotic and bacterial infections.

Materials and Methods: A female Humboldt penguin (*Spheniscus humboldti*) of unknown age from a private circus was submitted to multiple investigations at the Pathological Anatomy Department of the Faculty of Veterinary Medicine of Bucharest, after sudden death. Prior, clinical signs included anorexia, depression and progressive weight loss. The diagnostic methods included radiology, necropsy, cytology (M.G.G. stain), microbiology and histopathology (H.E., P.A.S. and Giemsa) examinations.

Results: Radiologic examination revealed discrete, focal, radiodense areas corresponding to the respiratory system.

At necropsy, the air sacs presented increased consistency. Multiple yellow, nodular lesions (1-10 mm) were observed in the air sacs and lungs and fewer nodules in liver, spleen and kidneys.

Imprint cytopathology revealed necrotic cells along with bacteria and mycetic hyphae. Microbiologic examination isolated *Aeromonas hydrophila* and *Candida krusei*.

Histopathology revealed both old and developing, multifocal granulomas, with peripheral multinucleated giant cells and cellular reactivity. Fungi were present both inside the granulomas and between lesions. Interstitial nephritis and glomerulosclerosis affected kidneys. The liver was affected by lymphohistiocytic, subacute inflammation and in spleen histiocytic infiltration and lymphocytic depletion were recognised. In addition, protozoan cysts (50-80µ in diameter) were identified in all major tissues.

Discussion and Conclusions: In Romania, this is the first communication concerning the pathology of a Humboldt penguin that died due to severe respiratory insufficiency. It is a typical example of death caused by synergic factors (bacteria, fungi and protozoa) in exotic captive animals.

Poster Presentations ESVP/ECVP

P103 PREVALENCE OF *BOVINE VIRAL DIARRHEA VIRUS* (BVDV) IN PERSISTENTLY INFECTED (PI) CATTLE AND EXPERIMENTAL SUPERINFECTION IN PI CALVES

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Introduction: *Bovine viral diarrhea virus* (BVDV), a *pestivirus* of the *Flaviviridae* family, is an economically important cattle pathogen with a worldwide distribution. In the present study, we used two donated naturally PI calves through survey studies (prevalence: 0.74%) and experimentally inoculated them with cp BVDV-1 using the IN or IV route, respectively.

Materials and Methods: Two male Korean native PI calves (calves 1, 2), which had been infected with ncp BVDV-1 naturally were superinfected experimentally with cp BVDV-1 intranasally (calf 1) or intravenously (calf 2). Clinicopathology, histopathology, immunochemistry (IHC), RT-PCR, and virus isolation were performed.

Results: Serum neutralising antibody against cp BVDV-1 was detected first at PID 5 and 9 and then developed a peak at PID 12 and 23 in calf 2 and calf 1, respectively. Gross and histopathological lesions were not prominent in calf 1, and the cp BVDV could not be isolated from samples from this calf. More severe and characteristic upper gastrointestinal lesions, including deep ulceration and lymphocytic inflammation in the tongue, erosion in the abomasal mucosa, and submucosal haemorrhage and congestion in the small intestine, were noted in calf 2 than in calf 1.

Discussion (and/or Conclusions): Not only the virulence, antigenic relationship, immunologic factors, and challenge dosage, but also the inoculation route could be an important factor in the experimental induction of MD in PI calves. To our knowledge, ours is the first report to compare the induction of MD via different inoculation routes using the BVDV isolated in Korea.

Notes:

P104 DEMONSTRATION OF MYCOBACTERIAL COMPONENTS IN CATTLE BY MOLECULAR AND CYTO-HISTOPATHOLOGICAL METHODS

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Introduction: The aim of the study was to to detect mycobacterial components in peripheral blood to provide an alternative diagnostic method for bovine tuberculosis (BTB) and also to compare the results of various diagnostic methods.

Materials and Methods: At slaughter blood and tissue samples, were collected from cows older than 2 years and previously found to be positive by intradermal tuberculin test (experiment group animals (EXA), *n*=30) and from clinically healthy cows younger than 2 years (control group (COA), *n*=30). The buffy-coat extracted from the blood by centrifugation was further processed with real-time PCR and the buffy-coat smears with immunocytochemistry (ICC) method. Real-time PCR in frozen tissues and HE, Ziehl-Neelsen (ZN) and immunohistochemistry (IHC) in formalin fixed, paraffin embedded tissues were performed.

Results: Tissues of 26 EXA in macroscopy, 28 EXA in HE, 25 EXA in IHC, 18 EXA in PCR; blood of 3 EXA in ICC, 14 EXA in PCR; blood of 10 COA in ICC and 2 COA in PCR were found to be consistent with BTB. Positive IHC staining was observed in the connective tissue surrounding the necrosis and intracytoplasmically in macrophages, giant-cells, rarely in lymphocytes. Positive ICC is observed intracytoplasmically in mononuclear leukocytes.

Discussion (and/or Conclusions): This study has shown the possibility of detecting the mycobacterial components in peripheral blood and the diagnostic prospect and importance of the blood in BTB. Various methods have been compared. IHC in tissue and PCR in blood samples were found to be the most effective method to diagnose BTB.

Notes:

Poster Presentations ESVP/ECVP

P105 *LAWSONIA INTRACELLULARIS* ENTERITIS IN TWO DANISH FOALS

Notes:

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Introduction: The obligate intracellular bacterium *Lawsonia intracellularis* causes adenomatous proliferation of infected enterocytes recognised as proliferative enteropathy (PE). PE is one of the most important intestinal diseases causing diarrhea, retarded growth and/or sudden death in pigs worldwide but is also found in other animals. In North America equine proliferative enteropathy (EPE) is well known especially affecting foals where it in some areas is seen almost epidemically. EPE has also been described from Europe (e.g. Belgium, Switzerland, Germany, and the Netherlands) but only sporadic. The aim of this study was to report two cases of EPE from Denmark.

Materials and Methods: Small intestinal segments from two 6 month old foals with a history of long standing diarrhoea, wasting, and subsequent death. Formalin-fixed, paraffin-embedded tissue samples were cut and stained by H&E. Identification of *L. intracellularis* was done by immunohistochemistry as well as by fluorescent *in situ* hybridisation.

Results: Grossly, hyperemia, increased thickness and rigidity of the intestinal wall characterised the segments from both foals. Histopathologically, typical PE lesions were widespread and *L. intracellularis* organisms were identified in enterocytes and macrophages by both *in situ* detection methods.

Discussion: The age of the two foals (6 months), gross and histopathologically lesions were typical for EPE and *L. intracellularis* organisms were detected by two independent methods. Both cases came from stud farms in Jutland with no contact to pigs but were otherwise not connected. To our knowledge this is the first cases of EPE reported from Denmark and Scandinavia.

P106 EXPERIMENTAL INFECTION OF GOATS WITH *MYCOBACTERIUM AVIUM* SUBSP. *HOMINISSUIS:* PATHOMORPHOLOGICAL INVESTIGATIONS

Notes:

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Introduction: *Mycobacterium avium* subsp. *hominissuis* (MAH) is an opportunistic pathogen, capable to establish granulomatous inflammation (GI) in several animal species and humans. The purpose of this study was to investigate the course of infection in goats under defined conditions.

Materials and Methods: Nineteen goat kids were orally inoculated with 10-times 10^8 bacterial wet weight MAH and 10 goat kids served as controls. Nine of the goats inoculated with MAH became emaciated up to 3 month after the first inoculation (mpi) and died spontaneously or had to be euthanised for animal welfare reasons. The remaining ten goats remained healthy and were euthanised at 13 mpi. At necropsy, macroscopic lesions were documented. Tissue specimens were collected for histology and bacterial culture. Lesions were investigated in HE-stained paraffin sections. MAH was labeled in paraffin sections by immunohistochemistry.

Results: Goats with early severe disease were kachectic and jejunal and ileal Peyer´s patches were extensively ulcerated. Mesenteric lymph nodes (MLN) were severely enlarged and calcified. Granulomatous infiltrates with necrosis and calcification were detected in gut associated lymphatic tissues and MLN. Numbers of MAH varied from single to numerous between animals. Animals necropsied 13 mpi were all in good condition, but small granulomas were present in IPP, large granulomas with extensive calcification in MLN and Peyer´s patches were atrophic.

Conclusion: MAH was capable to establish infection in all inoculated goats. Two different courses of disease developed: severe early disease with extensive granulomatous infiltrates and clinically inapparent infection with distinct encapsulated fibrotic granulomas.

Poster Presentations ESVP/ECVP

P107 *FASCIOLA HEPATICA* INDUCES APOPTOSIS OF INFLAMMATORY CELLS IN SHEEP AT DIFFERENT STAGES OF INFECTION

Notes:

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Introduction: Fasciolosis is an economically important disease of ruminants caused by *Fasciola hepatica* in temperate climates. *F. hepatica* has shown several mechanisms of immune evasion and immunomodulation to survive in the host, which difficult the development of protective vaccines. It has been reported that *F. hepatica* induces apoptosis of eosinophils and peritoneal macrophages in rats, but no studies have been conducted to elucidate if the parasite causes apoptosis in ruminant inflammatory cells and the importance of this phenomenon as mechanism of immune evasion.

Materials and Methods: Twelve 7-month-old *F. hepatica*-free sheep were used. Animals were divided into four groups (n=3 each). Groups 1, 2 and 3 were infected orally with 200 metacercariae of *F. hepatica* each. Group 1 was killed at 8 days post-infection (dpi), group 2 at 28 dpi and group 3 at 119 dpi. The group 4 was used as uninfected control. Apoptosis was investigated in liver tissue sections using an anti-caspase 3 antibody.

Results: Recent necrotic foci at 8 and 28 dpi showed numerous inflammatory cells (eosinophils, lymphocytes and macrophages) expressing caspase 3, whereas occasional caspase 3+ cells were found at the periphery of granulomas and portal inflammatory infiltrate at 28 and 119 dpi, where only some of the portal inflammatory infiltrated associated adult parasites showed numerous caspase 3+ inflammatory cells.

Discussion (and/or Conclusions): These results suggest that apoptosis may be an important mechanism of immune evasion of *F. hepatica* during the migratory stage of the infection.

P108 DETECTION OF *MYCOBACTERIUM AVIUM* SUBSP. *HOMINISSUIS* IN LYMPH NODES WITH- AND WITHOUT GROSS LESIONS FROM TUBERCULIN SKIN TEST POSITIVE PIGS

Notes:

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Introduction: *Mycobacterium avium* subsp. *hominissuis* belongs to MAC infecting mainly swine and humans. In one imported herd a high proportion of pigs reacted positively against avian and/or bovine tuberculin. As the gross and microscopic lesions were not correlated, mycobacteriosis and the possible source of infection were investigated.

Material and methods: Study was performed on 92 imported Norwegian Landrace swine that reacted positively against avian and/or bovine tuberculin. Formalin fixed, paraffin embedded mesenteric lymph node samples were analysed by real – time PCR for IS1245 specific for *M. avium* (MAC) and IS6110, specific for *M. tuberculosis* complex (MTC). In paraffin sections of all the mesenteric lymph nodes haematoxylin and eosin (HE) and Ziehl Neelsen staining were performed.

Results: Of the 92 (100%) samples analysed for IS1245 by real-time PCR, 51 (54.00%) were regarded positive. Ten (10.90%) of the 92 samples analysed by IS6110 real-time PCR were considered positive, all but three (3.26%) were simultaneously positive in IS1245 PCR. Of the eight pigs with neither microscopic nor macroscopic lesions only one sample was positive for IS1245, and none were positive for IS6110. Microscopically, granulomatous lymphadenitis was found in 84 of 92 examined mesenterial lymph nodes. In 30 (25.20%) cases of granulomatous lymphadenitis no macroscopically changes were established. Ziehl Neelsen positive rods were detected only in 5 (5.43%) of the samples.

Discussion and Conclusion: Identical and closely related isolates from peat and porcine lymph nodes were detected previously by RFLP analysis, indicating peat and sawdust as possible source of infection for the pigs.

Poster Presentations ESVP/ECVP

P109 DETERMINATION OF TELOMERASE HTERT EXPRESSION IN *BOVINE LEUKAEMIA VIRUS* (BLV) INFECTED CATTLE

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Introduction: Bovine leukaemia is characterised by a persistent lymphocytosis and B cell malignant lymphosarcoma development after extended latency periods. Telomerase catalytic subunit (hTERT) has been shown to play a critical role not only in telomere homeostasis but also in cellular survival, DNA repair, and genetic stability.The aim of the studies was determination of hTERT expression and telomerase activity in BLV infected animals.

Materials and Methods: Telomerase activity was analysed by Real-Time PCR in blood, lymphatic organs and dendritic cells of leukaemic and healthy cows. The telomere length and fluorescence intensity was determined with the use of fluorescence in situ hybridisation (FISH). The hTERT expression was investigated in immunofluorescence (IF) test with the use of monoclonal antibody.

Results: In all samples from BLV infected cows high activity of telomerase and expression of hTERT was found. The highest telomerase activity was detected in spleen and bone marrow, in cows with persistent lymphocytosis telomerase activity the highest in lymph node. The level of telomerase activity showed correlation with hTERT expression and telomere length.

Discussion (and/or Conclusions): Telomerase – a telomere – synthesing reverse transcriptase compensates the loss of telomere associated with cell division. hTERT encodes the catalytic subunit of telomerase and is present in most immortalised and cancer cells. Telomeres are important structures for the correct function and stability of chromosomes. Telomerase activity is expressed in most human tumour tissues, but not in normal tissues, except for those of the germline. Telomerase activity and hTERT expression almost always correlate with disease severity in lymphoproliferative disorders.

Notes:

P110 IMMUNOHISTOCHEMICAL STUDY OF CUTANEOUS IMMUNE RESPONSE AND KERATIN EXPRESSION IN GOATS WITH SARCOPTIC MANGE

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Introduction: Sarcoptic mange is one of the most important ectoparasitic diseases of goats in Greece. The aim of our study was to assess the cellular immune response and the expression of various cytokeratins in skin lesions of naturally infested goats with sarcoptic mange.

Materials and Methods: Selected cases of 40 goats with sarcoptic mange and 8 clinically healthy goats were studied. Human skin biopsies were also used as controls. Skin biopsies were fixed in zinc salts fixative. Tissue sections were immunostained against T-cell subpopulations (CD3, CD4, CD8, WC1 γδ TCR), B cells (CD21), dendritic cells (CD1b), macrophages (CD68) and cytokeratins (CKAE1/AE3, CKMNF116, CK34BE12, CK14, CK19, CK7 and CK5/6).

Results: The study of lymphocytic infiltrate showed a predominance of the CD3+ subpopulation (169.94±39.13 cells/mm^2) while CD21+ cells were sparse. In the dermal infiltrate, a predominance of CD4+ (91.95 ± 24.41 cells/mm^2) over CD8+ T cells (22.8±9.33 cells/mm^2) was observed. The CD4/CD8 ratio of lesional skin was 4.03±1.54/1. The γδ+ lymphocytes were expressed significantly (34.20±9.70 cells/mm^2) in dermis. Moreover, numerous CD1b+ and CD68+ cells were found in dermis, especially perivascularly. In scabietic skin, as the degree of epidermal hyperplasia increased, there was an altered expression of CKMNF116, CK19, and CK14 in most layers of the suprabasal epithelium and the outer root sheath of hair follicles. No difference was observed in immunostaining of CK5/6, CKAE1/AE3, CK34BE12 and CK7.

Discussion: The findings of our study emphasise that sarcoptic mange infestation stimulates the upregulation of antigen-presenting cells and T-lymphocyte subpopulations and alters the expression of certain cytokeratins as well.

Notes:

Poster Presentations ESVP/ECVP

P111 *ASPERGILLUS FUMIGATUS* INFECTION IN A COMPLICATED CASE OF BILATERAL PROGRESSIVE EQUINE ETHMOID HAEMATOMAS

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Introduction: Sinonasal surgery in horses may cause secondary nasal and/or sinus mycosis. Here we report a severe and infiltrating *Aspergillus fumigatus* infection in the nasal cavity and maxillary sinuses of a horse following surgical and formalin treatment of bilateral progressive ethmoid haematomas (PEH). In this case, antigen detection was attempted locally (immunohistochemistry) and systemically (ELISA) in order to state the diagnosis.

Materials and Methods: A 15-year-old female Oldenburg horse presented with a history of unilateral epistaxis and nasal discharge during several years. The diagnosis of bilateral progressive ethmoid haematomas was made endoscopically. After partial surgical removal of the masses from the right side via frontonasal sinus flap surgery and injection of formalin into the remaining haematomas, mycotic rhinitis and maxillary sinusitis developed bilaterally with fistulation through the right maxillary bone plate. Post mortem, necropsy as well as histological, immunohistochemical and mycological analyses were performed. Serum samples taken before and during (n=4) the infection period, were analysed for *Aspergillus* antigen (galactomannan).

Results: Necropsy revealed haematomas protruding into the sphenopalatine sinuses and the nasal conchae, as well as atrophy of the right ethmoid, mucopurulent fistulating maxillary sinusitis and mucosal fungal growth in the right maxillary sinus. Histological and immunohistochemical examinations revealed mucosal invasion of *A. fumigatus,* with conidia and conidiophores in the right maxillary sinus. The serum galactomannan index was negative throughout the entire infection period.

Discussion (and/or Conclusions): A well-documented equine case of invasive aspergillosis was serum galactomannan negative, which may be due to its localised nature within the upper airways.

Notes:

P112 *SCHMALLENBERG VIRUS* EXPERIMENTAL INFECTION ON PREGNANT GOATS

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Introduction: *Schmallenberg virus* (SBV) is an emerging virus in Europe, responsible for congenital malformations in domestic ruminants. This study was designed to look for the susceptibility window of the pregnant goat to the virus.

Materials and Methods: 14 pregnant goats were allotted into 3 groups. The goats of group A (5 animals) and group B (5 animals) were inoculated subcutaneously with SBV-infected serum at day 28 and day 42 of gestation respectively. The group C (4 goats) was the mock group. qRT-PCR and ELISA were performed on blood samples to evidence viraemia and seroconversion. At days 53 to 56 of gestation all animals were euthanised and submitted to necropsy. Maternal and fetal tissue samples were collected for qRT-PCR, histopathology and immunohistochemistry.

Results: All inoculated goats from groups A and B developed viraemia between day 3 and day 5 after inoculation and showed SBV-specific seroconversion from day 14 p.i. onwards. No clinical sign of infection was recorded. At necropsy, 5/11 fetuses in group A and 5/11 fetuses in group B were small and autolysed, indicating embryonic and/or fetal death. The other fetuses did not show any macroscopic anomaly. qRT-PCR results showed a broad range of SBV-positive organs in fetuses from infected goats.

Discussion (and/or Conclusions): The pregnant goat is susceptible to SBV at least between day 28 and day 42 of gestation. This study revealed for the first time a lethal effect of SBV in goat fetuses. The virus can widely disseminate in fetal organs.

Notes:

Poster Presentations ESVP/ECVP

P113 WATER-FILTERED INFRARED A (WIRA) IN COMBINATION WITH VISIBLE LIGHT (VIS) INHIBITS ACUTE CHLAMYDIAL INFECTION

Notes:

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Introduction: Water-filtered infrared A (wIRA) is short wavelength infrared radiation with a spectrum ranging from 780 to 1400 nm. In clinical settings, wIRA alone or in combination with visible light (VIS) has proven efficacy in acute and chronic wound healing.

Materials and Methods: HeLa and Vero cells were infected with either *Chlamydia (C.) pecorum* or *C. trachomatis*. *Chlamydia*-infected monolayers were irradiated with either a single dose of wIRA in combination with VIS (wIRA/VIS) at 40 hours post infection (hpi) or irradiated three times at 24, 36 and 40 hpi (triple dose). Non-irradiated, *Chlamydia*-infected cells were used as controls. The number and morphology of chlamydial inclusions was analysed using indirect immunofluorescence, titration by sub-passage and electron microscopy. Cytokine and chemokine release was analysed using commercially available ProteomeProfiler™ antibody arrays.

Results: A single dose of wIRA/VIS at 40 hpi significantly reduced chlamydial inclusion frequency regardless of the cell line or the chlamydial strain. Ultrastructurally, chlamydial inclusions had a normal morphology. A triple dose of irradiation further reduced the number of inclusions. Furthermore, chlamydial infection, irradiation or the combination of both triggered a similar release of pro-inflammatory cytokines (MIF/GIF, Serpin E1, RANTES, IL-6, IL-8) and chemokines (IL-16, IP-10, ENA-78, MIG, MIP-1α/β). Initial investigation of the working mechanism indicated possible thermal effects on *Chlamydia* due to irradiation.

Discussion: This is the first study to demonstrate a non-chemical reduction of acute chlamydial infection using the combination of water-filtered infrared A irradiation and visible light.

P114 ARENAVIRUS ASSOCIATED INCLUSIONS IN A PERIPHERAL ODONTOGENIC FIBROMYXOMA IN A RED-TAIL BOA

Notes:

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Introduction: Inclusion Body Disease (IBD) is the most important multisystemic viral infection affecting boas and pythons. Recently Arenaviruses have been put forward as the aetiological agents of IBD. A captive bred red-tail boa presented a large intra-oral mass originating from the buccal gingiva, attached to the dentary teeth row. A peripheral odontogenic fibromyxoma was diagnosed. Two years after removal, the neoplastic mass recurred.

Materials and methods: Sections of the mass and liver biopsy were collected for histopathologic evaluation. Whole blood was collected and HE stained buffy coat sections were prepared. Arenavirus RT-PCR was performed on whole blood, liver biopsy and the neoplastic mass.

Results: Histologically, the recurrent fibromyxoma consisted of spindle shaped cells embedded within a loosely arranged fibromyxomous stroma. The stroma contained collagen fibers as confirmed on Van Gieson staining and acid mucopolysaccharides as shown by alcian blue staining. The tumour cells contained oval nuclei with a fine granular chromatin and an eosinophilic fibrous cytoplasm. Immunohistochemical staining showed the tumour cells were vimentin positive. Approximately 50% of the tumour cell nuclei were positively labelled with Proliferating cell nuclear antigen (PCNA). Numerous large inclusion bodies, compatible with those observed in IBD affected snakes, were present in the neoplastic cells. Sections of liver biopsies and circulating lymphocytes, however, contained relatively few IBD inclusions. Using RT-PCR, Arenavirus was detected in blood, liver biopsy and neoplastic tissue.

Conclusion: The present case describes the co-occurrence of an Arenavirus infection and an odontogenic fibromyxoma in a red-tail boa.

Poster Presentations ESVP/ECVP

P115 INTESTINAL MICROSPORIDIOSIS IN A BEARDED DRAGON (*POGONA VITTICEPS*)

Notes:

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Introduction: A 4-month-old bearded dragon (*Pogona vitticeps*) was presented with a one-week history of anorexia, weight loss and severe depression. On physical examination, a large mass was palpated in the caudal coelomic cavity. Possible differential diagnoses included neoplasia, localised inflammatory process and foreign body. The animal was euthanised and grossly examined by the submitting veterinarian. The only gross lesion found was a mass in the colon which was sent for histological examination.

Materials and Methods: Histological and electron microscopic examination of the colonic mass was performed. Histological stains performed included Haematoxylin Eosin (HE), Periodic Acid-Schiff (PAS), Gram and Ziehl Neelsen (ZN).

Results: The colon wall was grossly focally massively thickened by a large (2 x 2 x 3 cm) tan firm multinodular mass. Histological examination revealed a severe focally extensive transmural granulomatous and necrotising colitis with numerous intrahistiocytic protozoal organisms which stained Gram positive and did not stain with ZN stain. A PAS stain revealed PAS-positive polar granules. By electron microscopic examination, the organisms revealed morphology consistent with *Encephalitozoon* spp., characterised by unikaryotic spores of 1.0-1.5 x 2.0-2.5 µm with a single row of 5-7 filament coils.

Conclusions: Microsporidia can infect a wide host range of invertebrates and vertebrates, including man. Microsporidiosis in reptiles, however, is only rarely reported in the tuatara, different snake species, African skinks, common wall lizard, and five inland bearded dragons. This is the first description of a non-systemic intestinal micropsoridiosis in a bearded dragon. Microsporidiosis should be considered a differential diagnosis in any sporadic granulomatous disease in reptiles.

P116 PATHOLOGICAL FINDINGS IN THE LUNGS OF REPTILES KEPT INDOORS UNDER UNSUITABLE ENVIRONMENTAL CONDITIONS

Notes:

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Introduction: Over the last years the popularity of reptiles as domestic pets has considerably increased. However, knowledge about proper husbandry is inadequate, which leads to an increase in the number of sick animals and often to their death.

Materials and Methods: Reptiles (n = 129) representing e.g *Iguana* sp, *Pogona* spp., *Gekko* sp., *Testudo* spp., and several species of the families Boidae and Pythonidae came from Polish and Czech private breeders. They were necropsied as a part of a larger study on the pathology of captive reptiles. At dissection, samples of the lungs and several other tissues were collected, fixed in a 4% neutral buffered formaldehyde solution, and embedded in paraffin. The sections were stained with HE, Azan Trichrome staining, and alcian blue-periodic acid-Schiff's reagent-haematoxylin (Alcian Blue-PAS, PAB).

Results: The autopsy revealed overal deteriorated condition of the majority of the animals. In addition to changes in other organs, hyperaemia, oedema, and various types of inflammation of the lungs were noticed. The presence of abscesses and granulomas in the lungs, mediastinum, and pleural cavity was also observed. Histopathology additionally showed infiltration of various cells in lung tissue.

Conclusions: In the light of these results, it seems essential to spread the knowledge about proper husbandry and breeding of exotic animals in captivity in order to reduce morbidity. It is also appropriate to train veterinarians (clinicians and pathologists) towards the diagnosis and treatment of diseases of exotic animals.

Poster Presentations ESVP/ECVP

P117 FIRST REPORT OF OPPORTUNISTIC BACTERIAL AND FUNGAL INFECTION IN TWO CASES OF SEPTICAEMIC CAPTIVE ALLIGATORS (*Alligator mississippiensis*) IN ROMANIA

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Introduction: According to the published literature, various ubiquitous fungal and bacterial microorganisms are involved as aetiologic agents in captive alligators. This study reports two cases of *Alligator mississippiensis* with bacterial and fungal disease.

Materials and Methods: Two adult American alligators, male and female, were submitted for necropsy. Post mortem X-ray examination, necropsy, cytology (MGG), histopathology (HE, HEA, PAS, Gram, Giemsa) were performed. Pleural and pericardial swabs were subjected to a microbiological examination.

Results: Grossly, the main lesions involved the lower respiratory system and were characterised by thoracal serosanguineous effusion, pleural and pulmonary nodules (1–40 mm, also observed radiologically) and accompanied by pulmonary oedema. Similar nodules also affected the liver, spleen and myocardium, suggesting a systemic disease. Additionally, cutaneous ulcers, gingival and gastrointestinal ulcers, unilateral enophthalmos and keratitis were revealed. Microscopical findings in major organs show lymphoid depletion and multifocal to coalescing necrotic areas with aggregates of coccoid and rod shaped bacteria intermixing fungal structures, boarded by a heterogenous inflammatory infiltrate, composed of epithelioid macrophages, lymphocytes and rare heterophils. The microbiological examination yielded growths of *Aeromonas hydrophila*, *A. caviae, Serratia marcescens, Pantoea agglomerans*, *Proteus vulgaris*, haemolytic and non-haemolytic *E. coli*, *Citrobacter freundii*, *Rhizopus*/*Absidia* from pleural and pericardial cavities.

Discussion and Conclusions: The death of the alligators was caused by the bacterial and fungal pneumonia, with secondary spreading of microorganisms, which developed a systemic disease. Environmental temperature changes, water quality or stress along with the low immune response are often listed as predisposing factors for developing these lesions due to opportunistic agents.

Notes:

P118 KERATINISING BASAL CELL CARCINOMA IN A SPUR-THIGHED TORTOISE (*TESTUDO GRAECA*)

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Introduction: A 31-year-old spur-thighed tortoise was presented with a slowly growing, 1,5 centimeter diameter, firm, white to pink, unencapsulated mass located at the interchoanal region.

Materials and Methods: A CT-scan was performed to assess the extensiveness of the mass. After partial surgical excision, samples were routinely processed for histopathology.

Results: Since the mass was located at the insertion of the right musculus adductor mandibulae, and was extending into the oral cavity, the tortoise had difficulties with food intake. No other clinical symptoms were diagnosed. The CT-scan showed deformation and destruction of the maxilla and *arcus zygomaticus*, as well as infiltrative growth in the retrobulbar region and masseter muscle. Histopathological examination revealed presence of a cell-rich, unencapsulated, multilobulated, expansile growing epithelial mass. The cuboidal cells were organised in nests and lobuli, had a scant amount of basophilic cytoplasm and a central round to oval, large nucleus with finely coarsed chromatin and 1-2 prominent nucleoli. There was mild anisocytosis and anisokaryosis. The mitotic index was 2-3 per high power field. Multifocally there was keratinisation centrally in the epithelial nests. Based on histopathology, a diagnosis of keratinising basal cell carcinoma was made.

Conclusion: Although basal cell carcinoma is the most common tumour in humans, the tumours are uncommon in cats and rare in dogs. This is the first case of a basal cell carcinoma in a tortoise. Currently, the mass has been removed three times as soon as the tortoise was suffering from mechanical interference with food intake. Complete excision is impossible.

Notes:

Poster Presentations ESVP/ECVP

P119 CHARACTERISATION OF SPLENO-PANCREATIC TUMOUR AND METASTASIS IN *BOTHROPS PUBESCENS* (REPTILIA, VIPERIDAE)

Notes:

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Introduction: The occurrence of neoplasms in reptiles has been increasingly reported, mainly due to the growing interest in wildlife by public and private institutions that use captive snakes for collections, anti-venom preparation, medical and scientific research or teaching.

Materials and Methods: An individual of "Pampas lancehead" (*Bothrops pubescens*) belonging to the Serpentarium of Venomous Snakes (UdelaR, Uruguay), presenting a large deformation that compromised the caudal half of the body, with clinical signs as anorexia and locomotor difficulty, was euthanised. The post-mortem examination revealed multiple intracoelomic tumours in stomach wall, spleno-pancreas, and four other masses adhered to posterior trunk vertebrae region and metastatic tumours in kidneys. Representative samples of tumours and organs were processed for histopathology (HE stain), immunohistochemistry (IHC) and Transmission Electron Microscopy (TEM).

Results: HE stain showed that neoplastic tissue was composed of fusiform cells with unencapsulated storiform pattern, supported by a fine fibrovascular stroma. The neoplastic cells had abundant intracytoplasmic vacuoles, nuclei were oval and elongated, with finely stippled chromatin and a single nucleolus. Mitoses were rare. IHC showed no immunoreaction against desmin, vimentin, and factor VIII. TEM of neoplastic cells showed bizarre forms with many intracellular vacuoles.

Conclusion: According to our preliminary results we propose that the primary tumour had an epithelial origin in the spleno-pancreas, and that the other tumours were metastatic.

P120 DETECTION OF A NOVEL NIDOVIRUS IN AN INDIAN PYTHON (*PYTHON MOLURUS*)

Notes:

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Introduction: Respiratory diseases frequently occur in captive pythons and can be caused by a large variety of infectious agents including viruses such as arenaviruses or paramyxoviruses. Members of the order of *Nidovirales* have been reported to cause disease in a large variety of animal species and humans. Members of this order have been detected in birds and mammalian species, but so far no nidoviruses have been described in reptiles.

Materials and Methods: A captive Indian python (*Python molurus*) was submitted for necropsy after sudden death. A full macroscopical and histological investigation was performed and lung samples were examined microbiologically. Moreover, random PCR in combination with 454 deep sequencing was used to identify a viral agent. Subsequent *in situ* hybridisation was used to detect intralesional viral RNA.

Results: Pathomorphological investigation revealed severe pulmonary and pancreatic necrosis. Salmonella and Bordetella species were detected in the lung samples. Using random PCR, sequences that were most closely related to viruses belonging to the subfamily *Torovirinae* were identified. Additional analysis revealed the complete genome of a novel nidovirus. Subsequent *in situ* hybridisation proved evidence of viral RNA within necrotic and viable lung epithelial cells.

Conclusions: This is the first description of an infection with the first member of *Torovirinae*, a novel nidovirus in a python, which is associated with necrotising pneumonia. It could thus play a role as an emerging pathogen itself, but also as an agent predisposing pythons to secondary bacterial infections.

Poster Presentations ESVP/ECVP

P121 INFECTION BY *ICHTHYOPHONUS*-LIKE PARASITES IN A NEOTROPICAL AMPHIBIAN

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Introduction: Parasites belonging to the Class Mesomycetozoea are amongst those pathogens that have been associated to the recent amphibian decline crisis worldwide. Most relevant mesomycetozoean parasites of postmetamorphic amphibians belong to the Orders Dermocystida (*Amphibiocystidium* and *Amphibiothecum*) and Ichthyophonida (*Ichthyophonus*). Dermocystids are currently known from amphibian hosts in Europe, North and South America, while *Ichthyophonus* has been reported in amphibians native to United States and Canada. In this work we communicate the occurrence of infection by *Ichthyophonus*-like parasites for the first time in a Neotropical amphiban.

Materials and Methods: A juvenile specimen of the arboreal frog *Hypsiboas pulchellus* (Hylidae) presenting a rounded swelling in the back was collected in southern Uruguay at Arroyo Mauricio, San José Department (34°40'S, 56°41'W), on November 2, 2007.

Results: Histological examination of the lesion revealed the presence of hundreds of capsulated cysts of approximately 100-200 µm. The cysts showed different degrees of development; some of them were in a resting condition whereas in others thousands of small spores of about 1 µm were fairly evident. No other specimens were collected at the study site, where *H. pulchellus* is common and abundant.

Discusion: Infection by *Ichthyophonus* was first reported in American Bullfrogs in 1953 and a bullfrog farm was still active close to the study site when the affected specimen was collected. Further field and molecular studies are needed to assess the extent to which these parasites may affect native amphibian populations, and wheter these parasites were introduced by bullfrogs.

Notes:

P122 *IN VITRO* INFLUENCE OF IRIDOVIRUS ON THE MACROPHAGE AND LYMPHOCYTE ACTIVITY IN STURGEON (*ACIPENSERIDAE*)

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Introduction: Iridoviruses, large double-stranded cytoplasmic DNA viruses are found in fish, amphibians and reptiles. Many new iridoviruses-like agents have been described in different species of fish. Their morphological and biological characteristics clearly separate them from the fish iridovirus associated with lymphocyctis disease, epizootic haematopoietic necrosis and epidermal infections. The mechanisms involved in the iridovirus penetration into fish are not understood. This study reports the preliminary results of the *in vitro* effects of iridovirus on the macrophage and lymphocyte activity in bester (*Huso huso* L. x *Acipenser ruthenus* L.) and *Acipenser gueldenstaedti* Brandt hybrids.

Materials and Methods: The pronephros and spleen were isolated and cells cultures were performed from 20 healthy sturgeons. The cells from pronephros and spleen were isolated on Histopaque-1077. The iridovirus (IFI-Poland) was quantified by plaque assay using the EPC cells incubated at 20 °C for 72 h. The metabolic activity (RBA) and potential killing activity (PKA) of spleen macrophages were examined. Also the proliferative response of pronephros lymphocytes stimulated by mitogens ConA and LPS were analysed.

Results: The results showed that iridovirus significantly decreased the macrophage activity and their potential killing activity in two examined species of sturgeon. The proliferative response of pronephros lymphocytes showed a similar pattern. Also the higher suppressive effect was observed in bester, compared to *Acipenser gueldenstaedti* Brandt hybrids.

Conclusions: This study demonstrated a strong inhibitory influence of iridovirus on the spleen phagocytes and pronephros lymphocytes in two species of sturgeon and suggested that the iridovirus induced a suppressive influence on cell-mediated immunity in sturgeon.

Notes:

Poster Presentations ESVP/ECVP

P123 PATHOGENESIS OF *CYPRINID HERPESVIRUS-3* (CYHV-3): IN VITRO EFFECT ON LYSOZYME ACTIVITY AND CYTOKINE-LIKE PROTEIN PRODUCTION IN CARP (*CYPRINUS CARPIO*), EUROPEAN CATFISH (*SILURUS GLANIS*) AND TENCH (*TINCA TINCA*)

Notes:

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Introduction: CyHV-3 has been isolated or identified from common carp and induced mass mortality in many countries. Clinical signs of CyHV-3 are often nonspecific and mortality may occur rapidly between 18 to 22°C. Several closely-related species cultivated with common carp: tench (*Tinca tinca*) or european catfish (*Silurus glanis*) in similar farms and sometimes in similar ponds did not present any symptoms of disease and were found to be fully resistant to it.

Materials and Methods: This study present the *in vitro* effects of CyHV-3 on the lysozyme activity in serum and interleukin 1-like protein and interleukin 6 – like protein productions by mitogen-stimulated spleen macrophages in carp, european catfish and tench. The blood and spleen were isolated and cell culture was performed on 10 healthy carp, tench and european catfish. Single cell suspensions were isolated and distributed in 24-well plates at concentration $1x10^6$ cells/ml of medium with 10% FCS and incubated 1 h with: ConA, ConA+CyHV-3 and only CyHV-3 at 20°C. The levels of IL-1-like and IL-6-like proteins were evaluated using ELISA kit assay and lysozyme activity by turbidimetric assay.

Results: The results showed that CyHV-3 significantly decreased the lysozyme activity in serum of carp, but not in tench and european catfish. Analysis of the results shows significant differences production of IL-1 and IL-6-like protein between cells isolated from carp, sheatfish and tench. The lowest levels of IL-1 and IL-6-like protein in carp were observed, compared to the European catfish and tench.

Conclusions: The CyHV-3 significantly reduced the cytokine production by spleen macrophages in carp.

P124 IMMUNOPATHOGENESIS OF HERPESVIRUSES: INFLUENCE OF *CYPRINID HERPESVIRUS-3* ON IMMUNOCOMPETENCE CELLS ACTIVITY – *IN VITRO* COMPARATIVE STUDY IN FISH

Notes:

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Introduction: Interest in lower vertebrate viruses has recently increased for a variety of reasons despite the fact that none of them infect warm-blooded animals and man. Some of lower vertebrate viruses have been used as models for studying disease mechanisms. The family *Herpesviridae* contains the three subfamilies: *Alphaherpesvirinae, Betaherpesvirinae, Gammaherpesvirinae* and are the most extensively studied fish viruses. First of all, because they are ubiquitous in aquatic organisms, since they have been isolated all over the world from both marine and freshwater fishes of different species. They are responsible for severe losses in aquaculture. Several viruses isolated from different species of fish have been classified as Herpesviruses. Several DNA viruses infecting fish have been studied for analysis the pathogenesis and for developing the effective methods of prevention and therapy. Also some of fish viruses have been used as models for studying disease mechanisms.

Materials and Methods: In our study we present the *in vitro* influence of *Cyprinid herpesvirus-3* (CyHV-3) on the phagocytic ability and potential killing activity of splenic phagocytes and proliferative response of pronephric lymphocytes stimulated by mitogens ConA and LPS isolated from carp (*Cyprinus carpio*) and tench (*Tinca tinca*).

Results: The results showed that CyHV-3 induced a suppressive effect on the phagocytic ability and potential killing activity of splenic phagocytes and decreased the lymphocyte T and B proliferation isolated from pronephros in carp, compared to the tench where the immunostimulating effect was observed.

Conclusions: This study suggested that CyHV-3 inhibited cell-mediated immunity in carp.

Poster Presentations ESVP/ECVP

P125 AETIOLOGY AND PATHOGENESIS OF ATYPICAL BACTERIAL GILL DISEASE (ABGD) IN SALMONIDS

Notes:

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Introduction: ABGD induces high mortality in intensive Salmonids culture. The pathogen can be cultivated from diseased tissue on low-nutrient media by incubating at 15°C. The pathogen is a G-, yellow-pigmented bacterium that is strongly proteolytic. A number of extracellular toxins and enzymes, which are associated with the virulence of other fish pathogens, have been described for *Flavobacterium psychrophilum*. Many experimental studies suggest that bacteria have a varied pathogenicity that depends on fish size and water temperature. The present study examined the influence of the new ABGD on the innate immunity in fingerling of rainbow trout.

Materials and Methods: The fish of 10-20 g body weight were examined after low levels of mortality at temperature of 10 °C were observed. Dystrophic changes were observed only in the gills. The bacteriological, mycological and virological study showed that only *Flavobacterium psychrophilum* was isolated from gills. Circulating blood was collected from fish in the first day when the symptoms appeared. Other samples were taken after 3, 7 and 10 days from 20 affeccted fish and 20 unafflected animals to examine the innate immunity.

Results: The results showed that phagocytic ability (RBA) and potential killing activity of spleen macrophages were statistically ($P<0.05$) lower in fish with disease syndrome in compared with disease-free fish. Also the lysozyme activity and Ig levels in serum were significantly lower compared to healthy fish.

Conclusions: The results indicated that the suppression occurred as atypical bacterial gill disease was developing. This preliminary study indicated that *F. psychrophilum* has a strong suppressive influence on cellular and humoral immunity.

P126 MORPHOLOGICAL STUDIES ANSWER THE QUESTION WHY THE REARING OF THE RAINBOW TROUT (*ONCORHYNCHUS MYKISS WALBAUM, 1792*) NEEDS INNOVATIVE SOLUTIONS

Notes:

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Introduction: The morphological examination may be used as a tool for monitoring the health condition of fish.

Materials and Methods: The studies were conducted between 2010 and 2012 in the spring and autumn in 6 rainbow trout farms: 3 with a flow through system (FTS) – on 480 rainbow trouts (group 1) and 3 with a recirculation aquaculture system (RAS) – on 480 trouts (group 2). Liver was collected for studies. Sections of liver were stained with HE. Material for ultrastructural examinations was contrast-stained with 0,5% uranyl acetate and lead citrate in Ringer solution.

Results: Macroscopically lesions of skin and scales were observed (more frequent in the group 2). Microscopic analysis of the liver of the rainbow trouts showed in the majority of the fish the normal pattern (independently from the body mass and rearing system). Steatosis simplex and hyperaemia were found comparatively often, especially in the group 2. Quantitatively, more lesions were found in the fish of the group 2, however the difference was statistically insignificant. Ultrastructural studies showed comparatively often small alterations of the mitochondrial structure (oedema, dense bodies), sporadically the loss of cristae structure and matrix rarefication. Seldom alterations were concerning the rough endoplasmic reticulum.

Discussion: The extent of liver lesions was higher in the fish from the intensive rearing system. The obtained pattern allows to claim that the most sensitive issue of the RAS technology is assurance of cellular respiration – the appropriate water temperature and oxygen concentration. It makes the water oxygen concentration monitoring necessary.

Poster Presentations ESVP/ECVP

P127 MELANOMACROPHAGES ACCUMULATION IN THE LIVER, SPLEEN AND KIDNEY OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS* WALBAUM, 1792) AS A TOOL FOR THE ASSESSMENT OF THE ENVIRONMENTAL INFLUENCE ON FISH

Notes:

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Introduction: Macrophage aggregates called melanomacrophage centres (MMCs) increase in size or frequency in conditions of environmental stress and have been suggested as reliable biomarkers. The aim of this study is histological analysis to investigate the influence of extensive and intensive rearing on MMC accumulation in the liver, kidney and spleen of rainbow trouts.

Materials and Methods: The studies were conducted during 2010-2012, in spring and autumn on 960 rainbow trouts. The specimens from liver, spleen and kidney of fish were collected in 3 farms with a flow-through system (FTS), extensive rearing, and in 3 farms with a recirculation aquaculture system (RAS), intensive rearing. Histological sections of these organs were stained with HE and observed to determine the rate of MMCs accumulation.

Results: In 5% of the examined trouts melanomacrophages were present in the liver. In the spleen they were present in 20-30% of trouts. MMCs were observed in every section of the anterior kidney. The posterior kidney was the most often affected organ by the melanomacrophage infiltration. They were observed usually as a vast gathering forming "centre". In every examined organ MMCs were more frequently observed in the fish coming from farms using RAS technology.

Discussion: Macrophages are a form of biological cleaning stations. Their presence in the fish organs is beneficial, but it also may indicate the level of environmental pollution. The melanomacrophage infiltration is related with the aging and stress associated with environmental xenobiotics. The results show that intensive rearing causes a greater accumulation of MMCs than FTS system.

P128 PATHOMORPHOLOGICAL EXAMINATION OF THE RAINBOW TROUT *(ONCORHYNCHUS MYKISS* WALBAUM, 1792) LIVER AS A TOOL FOR ASSESSMENT OF WATER QUALITY AND ITS INFLUENCE ON FISH CONDITION

Notes:

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Introduction: The aim of the study is to find the relation between health indices of trout reared in the freshwater aquaculture and results of the pathomorphological examination of the liver, biometric and qualitative analysis of water with advanced explorative statistics.

Materials and Methods: Fish for studies were collected in spring 2011 in two trout farms: OS – open flow-through system – 40 fish and RAS – water recirculation system – 40 fish. Macroscopical trout examination was based on the visual inspection and the examination of internal organs. Liver was subjected to microscopical pathomorphological (HE staining) and ultrastructural examination. Additionally, the following parameters were studied: breeding parameters – biometric, Fulton condition coefficient, in-flowing water quality indices. Statistical analysis was performed on the logarhythmic co-relation matrix. Principal Component Analysis (PCA), data clustering and multidimensional regression were used.

Results: On the basis of PCA analysis fish were assigned to 3 groups: A-C. In group C the more frequent lesions were: skin and scales lesions, steatosis simplex, hyperaemia and small mitochondrial changes. The first component clearly differentiates groups A and C. On the scatter diagram the overlap of points concerning groups A and B can be seen. It was shown that the parameters of inflowing water were most important for group assignment.

Discussion: The extent of liver changes was higher in fish from farms with lower water parameters. No influence of the technology (RAS, OS) on the fish condition was seen. The most sensitive point in trout rearing is assuring appropriate water temperature and oxygen concentration.

Poster Presentations ESVP/ECVP

P129 HPV-16-TRANSGENIC MICE AS MODELS TO STUDY PAPILLOMAVIRUS INDUCED LESIONS

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Introduction: Papillomaviruses are a cause of disease in animals, including several economically important syndromes. K14-HPV16 transgenic mice expressing the HPV16 early genes in squamous epithelia may provide a suitable experimental model for the study of these diseases.

Materials and Methods: A colony of K14-HPV16 mice was established by crossing hemizygous and wild type animals. Twenty one animals were genotyped using tail tip samples. Tissue lysis was performed using *MAGNAPure DNA Tissue Lysis Buffer* and Proteinase K for 17h at 65°C. Nucleic acids were extracted by the *High Pure Viral Nucleic Acid*. The integration of HPV was assessed by amplification of HPV-E6 and E2 genes by polymerase-chain-reaction methodology in-house. The genotypes were confirmed to the respective phenotypes. All animals were humanely sacrificed at 22 to 26 weeks-old.

Results: We observed the presence of integrated HPV DNA in 43% of mice. All cases with HPV-E6 DNA also presented HPV-E2 DNA sequences. All mice with integrated HPV DNA demonstrated, phenotypically, various degrees of epidermal and squamous mucosal hyperplasia and dysplasia. Ear skin showed *in situ* carcinoma, while oropharyngeal mucosa showed mild to moderate dysplasia. No histological changes were observed in the urinary bladder.

Discussion and Conclusions: K14-HPV16 mice show several lesions resembling those caused by BPV and COPV in cattle and dogs, respectively, and may be a useful model to study the diseases associated with these agents. The phenotype must always be complemented with the results of genotyping, especially in cases with doubtful phenotypes. Thus, the determination of offspring genotypes is essential for any study with K14-HPV16.

Notes:

P130 CO-EXISTING SYSTEMIC AND INTESTINAL NEOSPOROSIS IN A NATURALLY INFECTED DOG

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Introduction: The apicomplexan parasite *Neospora caninum* can cause morbidity and mortality in many species of animals, including dogs. The dogs with systemic neosporosis show meningoencephalitis, pneumonia, polymyositis and polyradiculoneuritis, while the intestinal form of the disease, only demonstrated in experimental studies, is asymptomatic.

Materials and Methods: A 1.5 month-old Kangal breed puppy that had lived at a dairy cattle ranch died after showing severe diarrhoea and incoordination. Tissue samples were processed with routine histopathology, and immunoperoxidase test and PCR were performed for *N. caninum*. Sera of the cattle (n=66) and dogs (n=7) in the ranch where the puppy originated were analysed using commercial c-ELISA and IFAT, respectively.

Results: At necropsy, consolidation and necroses in the lungs, and haemorrhagic-fibrinous enteritis were observed. Histopathologically, necrotic and purulent bronchopneumonia, myocarditis, and non-purulent meningoencephalitis were detected. In the ileum, villous atrophy, crypt hyperplasia and dense eosinophil leukocytes and macrophage infiltrations were present. In the intestinal mucosa and crypt epithelia, there were oocyst-like and schizont-like structures. Immunohistochemically, strong positive immunoreactions for *N.caninum* antigen were detected in degenerative neurons, necrotic areas in lungs, heart and intestinal developmental stages of the protozoan. PCR analyses also revealed positive *N.caninum* seroprevalance that was 74.2% (49/66) in cattle and 57.1% (4/7) in dogs.

Conclusions: In this field report, co-existence of intestinal stages of *N.caninum* and fatal systemic neosporosis has been reported in a naturally infected puppy. To the best of the authors' knowledge, this is the first definition of intestinal neosporosis in naturally infected dogs and represents the first evidence of fatal dog neosporosis in Turkey.

Notes:

Poster Presentations ESVP/ECVP

P131 VASCULAR HYPERTROPHY IN RELATION TO SCHISTOSOMIASIS IN A MUTE SWAN IN POLAND

Notes:

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Introduction: The mute swan (*Cygnus olor*) is known to be the final host of schistosomes. However, there is relatively little information about the effects of Schistosomatidae on their avian host.

Materials and Methods: One mute swan (*Cygnus olor*) male, immature was clinically examined (blood test, microbiology, parasitology) and finally was necropsied. Tissue samples were processed by histopathology (HE, van Gieson, Congo red, Perls), IHC (αSMA), PCR followed by sequencing.

Results: Avian schistosome infection with *Dendritobilharzia pulverulenta* was confirmed by PCR and sequencing (respectively with 99%, 93% sequence identity in Blast, GenBank: KJ438954.1). Necropsy examination revealed haemorrhagic-necrotic enteritis and emaciation. HP examination revealed a proliferation of venous fibers with obliteration of the lumen in mesentery, serosa and muscle layer of intestines. Adult schistosomes were noted in the serosal veins, schistosome eggs were present in intestinal villi. The proliferated venous fibers were αSMA positive. Granulomatous lesions, fatty degeneration and mild iron deposition in Kupffer cells were found in liver, glomerular lesion – in kidney. Amyloid deposition was noted in blood vessels of the spleen and heart.

Discussion (and/or Conclusions): To the authors' knowledge this is the first reported case of vascular hypertrophy associated with schistosomiasis in mute swan in Poland. This lesion may have contributed to the cachexia and death of mute swans by obstruction of the venous system.

P132 MYCOBACTERIOSIS IN THE BLUE-CROWNED HANGING PARROT (*Loriculus galgulus*) – A CASE REPORT

Notes:

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Introduction: The Blue-crowned Hanging Parrot (*Loriculus galgulus*) is a small parrot found in forested lowlands from Thailand to Borneo. In parrots, tuberculosis (TB) is mainly caused by *M. avium*, and less frequently *M. intracellulare* and *M. genovense*. Lesions are typically found in the liver and gastrointestinal tract, although many other organ systems can potentially be affected. Avian mycobacteriosis is an important disease which affects companion, captive exotic, wild and domestic birds. The disease is zoonotic.

Material and Methods: The Blue-crowned Hanging Parrot was a 3 year old female with clinical signs of depression and density of faecal lasting two days; died in the aviary. During necropsy all internal organs were taken to the histopathological examination. They were fixed and stained routinely (HE), moreover Perls, PAS and Ziehl – Neelsen method.

Results: Lesions were typically found in the gastrointestinal tract. The intestinal mucosa was severely thickened as a result of granulomatous inflammation. Very large numbers of epithelioid cells were present in the lamina propria. In ZN staining, the large number of acid fast bacilli. Apart from a few mucosal crypt remnants were present. At the necropsy, the liver was enlarged and dark-red in colour, the kidney were also congested. Histopathologically, iron-laden macrophages were scattered in the liver.

Conclusion: Gross pathology and microscopy showed infection with avian mycobacteria. To our knowledge, this is the first report of the incidence of mycobacteriosis in *Loriculus galgulus* noted in Poland.

Poster Presentations ESVP/ECVP

P133 PARTRIDGE DIAGNOSIS OF A SYNDROME SIMILAR TO MAREK'S DISEASE TRANSIENT PARALYSIS

Notes:

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Introduction: Marek's disease (MD) has been reported in several species such as chicken, quail, turkey and pheasant but to our knowledge never in partridge. In this work we report a disease similar to transient paralysis in partridge, a special form of MD previously described in chicken.

Materials and Methods: Different degrees of ataxia and flaccid paralysis of neck, wings and legs were observed in 4 month-old partridges on a farm with 14,000 birds. Around 7% were affected, with 70% mortality. Necropsies were performed on 12 partridges with several degrees of the disease. Histopathology (HE) studies were carried out and inmunohistochemistry (IHC) and/or PCR techniques were also made to detect *Mareks disease virus*.

Results: Brain (CNS) and peripheral nerves (PNS) were the most affected organs showing small and focal perivascular cuffs of mononuclear cells, pyknosis and neuronal shrinkage, demyelination, axonal swelling and oedema. Perivascular cuffs were also observed in liver. Positivity in CNS, PNS and liver was observed in IHC and PCR was also positive to *Marek's disease virus* presence.

Discussion and Conclusions: Lesions observed could be related to mild Marek's disease described in chicken and some of them could also be associated with transient paralysis syndrome. IHC and PCR results confirm Marek's disease diagnosis in partridge.

P134 HISTOPATHOLOGICAL STUDY OF THE LESIONS INDUCED BY SUBTYPE H9N2 AVIAN INFLUENZA VIRUS AND *ORNITOBACTERIUM RHINOTRACHEALE* IN SPECIFIC PATHOGEN FREE CHICKENS

Notes:

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Introduction: Although H9N2 subtype avian influenza virus (H9N2 AIV) is classified as a low pathogenic avian influenza virus (LPAI) there are many reports of fatality and economic losses, from 1990, associated with this subtype. It is likely that many factors/other microorganisms have a role in these unexpected reports. The aim of this study was to evaluate the histopathological lesions induced by coexisting infection of specific pathogen free (SPF) chickens with AIV subtype H9N2 and *Ornitobacterium rhinotracheale* (ORT).

Materials and Methods: Histopathologic examination was performed on different organs, collected on different days post inoculation (PI), of the SPF chickens (n=80) which were inoculated with H9N2 AIV and/or ORT.

Results: There was mortality (3/20) and more severe histopathologic lesions in different organs of the chickens infected, concurrently, with H9N2 AIV and ORT in comparison with other groups, which were infected solely by one of the microorganisms.

Discussion (and/or Conclusions): The data obtained from current study clearly showed that simultaneous infection of chickens with ORT and H9N2 AIV increases the virulence of the AIV which can lead to mortality and economic losses.

Poster Presentations ESVP/ECVP

P135 TISSUE DISTRIBUTION AND TROPISM OF PCV2 IN FIRST DETECTED CASES OF PORCINE CIRCOVIRUS ASSOCIATED DISEASE (PCVAD) OF WILD BOARS IN SERBIA

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Introduction: *Porcine circovirus type 2* (PCV2) affects both domestic swine and wild boars, causing PCVAD. There were no data on the presence of PCVAD in wild boars in Serbia.

Materials and Methods: PCR analysis was done on spleen samples of 100 young wild boars in two hunting areas. To investigate the PCV2 distribution and tropism in PCR positive cases, *in situ* hybridisation was performed on lymphoid and non-lymphoid organs, using complementary probe – detecting non-replicative form (nPCV2) and replicative probe-detecting replicative form (rPCV2).

Results: PCR analysis proved the PCV2 infection of 24 (24/100) wild boars in both hunting areas. Microscopically, severe lymphocyte depletion, macrophage accumulation and granulomas were present in lymphoid tissue. In four PCVAD affected wild boars a high amount of nPCV2 was detected and tropism (rPCV2) was proved for both lymphoid and non-lymphoid tissue. In the lymphoid tissue nPCV2 genome was predominantly detected in the cytoplasm of macrophages, while the rPCV2 was found occasionally in the nucleus of lymphocytes, rarely in macrophages. The nPCV2 and rPCV2 genome were also located in epithelial cells of the renal tubules and bile ducts, hepatocytes, bronchoalveolar epithelium, and enterocytes.

Conclusions: Despite the widespread occurrence of PCV2 infections in domestic pigs in Serbia, the data about PCVAD of wild boars were missing. The current study presents PCV2 tissue distribution and tropism in the first detected cases of PCVAD of wild boars in Serbia.

Notes:

P136 HISTOPATHOLOGY OF A SYSTEMIC *BAYLISASCARIS PROCYONIS* INFESTATION IN A WHITE-HEADED LEMUR (*EULEMUR ALBIFRONS*) IN NORTHERN SPAIN

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Introduction: We describe a case of fatal *larva migrans* by *Baylisascaris procyonis* in a captive white-headed lemur from a Zoological Park in Galicia, Spain. The animal showed signs of weakness, incoordination and head tilt a few days prior to death. *Baylisascaris procyonis* is a roundworm infection of raccoons (*Procyonis lotor*) that causes serious *larva migrans* in other hosts, including humans, with high tropism for the central nervous system.

Materials and Methods: Formalin-fixed tissue samples from all organs collected during necropsy were submitted to the Pathology Service of the Complutense University Veterinary Hospital for examination. Relevant tissues were shipped to Zoologix Inc. (Chatsworth, CA) for commercial PCR of *Baylisascaris procyonis*.

Results: Histopathology revealed granulomatous encephalitis with gliosis and malacia, and intralesional ascarid larvae. Remaining tissues had multifocal granulomatous pneumonia, nephritis, myocarditis, splenitis and hepatitis of variable severity and occasional larvae. PCR from paraffin-embedded cerebrum confirmed *Baylisascaris procyonis*.

Discussion (and/or Conclusions): Raccoons are autochthonous of North America, but recent importation of these animals and uncontrolled introduction to wildlife has led to the expansion of this species in other geographical regions, including Spain. However there were no records of the nematode *Baylisascaris procyonis* in this country until now. The zoological park where this lemur was held was in a region with a fair density of raccoons. This finding stresses the importance of controlling the spread of non-autochthonous species and forewarns against a new zoonotic agent of grave consequences in Spain.

Notes:

Poster Presentations ESVP/ECVP

P137 ACTIVITY OF NA+/K+-ATPASE IN CHICKENS AFTER ADMINISTRATION OF β-D-GLUCAN AND LOW DOSES OF T-2 TOXIN

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Introduction: The Na^+/K^+-ATPase is a key transport element required for the establishment of electrochemical gradient driving cellular transport and substrate flow across epithelia. In addition, the enzyme is involved in basic processes such as maintenance and proliferation. T-2 toxin in small doses can damage the mucosa of the digestive tract and impair resorption of nutrients. This toxic effect prompts us to follow epithelial localisation and activity of the Na^+/K^+-ATPase after interaction with β-D-glucan and low doses of T-2 toxin.

Materials and Methods: Day-old chickens (n=20) were divided into four groups: T-2 toxin (145 μg pure/1 kg diet; from 14 to 28 day of age), β-D-glucan (3 mg/chicken), β-D glucan+T-2 toxin, and controls. Immunohistochemistry using MoAb-specific for α subunit Na^+/K^+-ATPase and secondary antibody conjugated to Alexa Fluor 488 on mid jejunum samples fixed in 4% paraformaldehyde were done. Intensity of immunofluorescence was evaluated by NIS-Elements software (Nikon, Japan).

Results: The a subunit-specific anti-Na^+/K^+-ATPase antibody identified the protein in the cell membrane of jejunal enterocytes from the epithelial cells to *lamina propria mucosae* cells. Higher expression of Na^+/K^+-ATPase ($P<0.05$) was found in β-D glucan+T-2 toxin group comparing to other three groups. T2-toxin group showed tendency to increase expression of Na^+/K^+-ATPase comparing to β-D-glucan-group and controls.

Conclusions: Low doses of mycotoxin did not alter the activity of Na^+/K^+-ATPase. On the other hand, immunomodulator β-D-glucan in chickens fed diet contaminated with T-2 toxin increased activity of that enzyme. Evaluation of other morphological parameters is needed. Results demonstrated that evaluation of Na^+/K^+-ATPase can be successfully used in poultry.

Notes:

P138 A MICROSCOPIC GRADING SCHEME FOR BISPHOSPHONATE RELATED OSTEONECROSIS OF THE JAW USING A MINIPIG TOOTH EXTRACTION MODEL

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Introduction: Bisphosphonates (BPs) are well tolerated drugs to treat resorptive skeletal alterations (osteoporosis, Paget's disease, metastatic malignancies). However, since 2003 a rare but severe clinical condition called bisphosphonate-related osteonecrosis of the jaw (BRONJ) is observed. BRONJ is currently only defined by clinico-anamnestic features: (a) the presence of orally exposed bone for › 8 weeks, (b) a positive BP drug history, (c) no prior irradiation to the head-neck region. As this definition does not include any specific histopathological parameters we developed a scoring system using a minipig tooth extraction model.

Materials and Methods: In total, 12 Göttingen minipigs were assigned to 3 groups. Group 1 received BP for 19 weeks (0.05 mg/kg zoledronate [ZOL] i. v.). As a trigger, in group 2 four premolar teeth (PM) were extracted after 11 weeks of ZOL followed by another 8 weeks of ZOL. In group 3 only teeth were extracted. Following euthanasia and necropsy, histological analyses was performed on all PM_4 samples using non-decalcified, resin-embedded, Giemsa-Eosin-stained thick-sections.

Results: Changes were graded using a semiquantitative scoring scheme (1-5) and consisted in gingivitis, periodontitis, osteomyelitis, empyema of *canalis mandibularis* resp. *sinus maxillaris*, infection with Giemsa-positive bacteria (partly along with sulfur granules), reduced osteoblastic bone formation, increased osteoclastic bone resorption, osteonecrosis, osteolysis, and gingival ulceration.

Discussion (and/or Conclusions): The concurrent presence of two hallmark features, orally denuded bone (gingival ulceration) along with degradation of extracellular bone matrix (osteolysis), correlated to the current definition of BRONJ. The pathogenic importance of *Actinomyces* ssp., confirmed by pathognomonic Splendore-Hoeppli material, is unknown.

Notes:

Poster Presentations ESVP/ECVP

P139 A 13-WEEK GAVAGE TOXICOPATHOLOGICAL STUDY IN SPRAGUE-DAWLEY RATS WITH SP-8

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Introduction: The objective of this study is to evaluate the subchronic toxicity of SP-8 (a candidate compound developed to treat smallpox virus infection) in rats.

Materials and Methods: Four groups of Sprague-Dawley rats (n=30), aged 5-6 weeks, were administered SP-8 (purity≥99.5%) in the solvent of 0.75% of methyl cellulose containing 1% tween-80 by gavage at dose levels of 0, 45, 180, and 720 mg/kg body weight, 6 days per week for 13 weeks. The rats were necropsied at the end of week 6 (8/group) and 13 (14/group) post administration, and week 4 (8/group) post drug withdrawal, respectively. All major tissues were processed for histopathologic examination. Stains of Mallory-Heidenhain and immunohistochemistry of α2μ-globulin were performed on male and female rat kidney sections.

Results: The kidney-to-body ratios were significantly increased in the males in the MD group at week 6 and the males in the HD group at week 13. Three sets of lesions were found in the kidneys of the males in all three dosed groups at week 6, which exacerbated at week 13 and alleviated after 4 weeks of recovery: hyaline droplet accumulation in the cortex, granular casts at the junction of the outer and inner stripes of the outer medulla, and patchy tubule dilation or atrophy. Stains of Mallory-Heidenhain and immunohistochemistry of α2μ-globulin on the kidney sections of the males showed positive results.

Discussion (and/or Conclusions): SP-8 administration to Sprague-Dawley rats by gavage at dose levels of 45, 180, and 720 mg/kg body weight for 13 weeks could cause α2μ-globulin nephropathy.

Notes:

P140 MORPHOLOGICAL LESIONS IN MOUSE LIVER AFTER PULMONARY EXPOSURE TO MULTI-WALLED CARBON NANOTUBES (MWCNTs) OF DIFFERENT PROPERTIES

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Introduction: Although MWCNTs attract industrial interest due to their unique properties the knowledge about their potential adverse effects is still sparse. The effect of $MWCNT_{Small}$ (length 0.8±0.1 μm) or $MWCNT_{Large}$ (4±0.4 μm) on mouse liver morphology was compared.

Materials and Methods: The mice (female C57BL/6 aged 8 weeks) received 18, 54, 162 μg of $MWCNT_{Small}$ (A-C groups) or $MWCNT_{Large}$ (A1-C1) by single intratracheal instillation. The doses reflect 1, 3, 28 days of 8 h time weighted exposure limit for carbon black particles (3.5 mg/m^3) in an occupational setting in Denmark. Vehicle control animals (group D) received NanoPure water with 2% serum. Liver samples were taken on day 1, 3, 28 after instillation (a.i.). from 6 animals/group except for group B (n=5, 1 day a.i.) and were stained with HE.

Results: The vacuolar degeneration was observed in the centrilobular zone in A, mid-zonal in B and in the periportal zone in C. In the livers from groups A1-C1 this lesion was observed in the entire hepatic lobule. Eosinophilic necrotic foci were observed more frequently in livers from B1, C1 then from B/C. Similar group dependency was observed for granulomas and hypertrophy of Kupffer cells. The morphological changes were observed more intensive in mice exposure to $MWCNT_{Large}$. For both types of MWCNT increased incidence was observed in the livers on day 3, 28 than on day 1 a.i.

Conclusions: Pulmonary exposure to $MWCNT_{Large}$ was associated with increased incidence and severity of morphological liver lesions as compared to the exposure to $MWCNT_{Small}$.

Notes:

Poster Presentations ESVP/ECVP

P141 PRENATAL DEVELOPMENTAL TOXICITY STUDIES ON RODENTS AND NON-RODENTS PERFORMED IN INSTITUTE OF INDUSTRIAL ORGANIC CHEMISTRY IN PSZCZYNA – RECORDED CHANGES

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Introduction: The prenatal developmental toxicity study is performed in order to evaluate influence of tested item on pregnant females and developing fetuses and potential to induce structural and functional abnormalities in fetuses as well as increases in their mortality.

Materials and Methods: The studies were performed on rats and rabbits. In each study there were three treated groups and one control group of pregnant females. Pregnant females were treated from 5th to 19th day of gestation – rats, and from 6th to 27th day of gestation – rabbits. Clinical signs, body mass and food consumption were controlled. All females were subjected to caesarean section. The fetuses were examined for deformations of body and the skeletons of the fetuses were evaluated.

Results: The following changes were stated in rats' fetuses: statistically significant increase in average weight of fetuses, weight of placenta and lengths of fetuses in treated groups, supranumeral fingers (*polydactylia*), lack of fingers and wavy ribs. The following changes were stated in rabbits: increased number of resorptions, statistically significant lower average number of ossification points in metacarpus, 7 fingers (*polydactylia*) in forelimb and conjoined fetus.

Discussion (and/or Conclusions): The following changes: *polydactylia* in rats, conjoined fetus and *polydactylia* in rabbits give evidence of teratogenic effect. Conducting this type of studies on two species makes it possible to confirm a non-random character of the observed changes and to facilitate determination of potential risk to humans.

Notes:

P142 EFFECTS OF TRIHALOMETHANES ON LIVER MITOCHONDRIA

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Introduction: Trihalomethanes (THMs), namely dibromochloromethane (DBCM) and bromodichloromethane (BDCM), are disinfection byproducts of chlorinated water. This experiment aimed to evaluate the mitochondrial dysfunction induced by THMs at low levels in a mouse model.

Materials and Methods: Experimental procedures were made in accordance to the European Directive 2010/63/EU. Forty-two ICR male mice were randomly divided into 4 experimental groups: DBCM-exposed (n=11), BDCM-exposed (n=11), methanol-exposed (n=11) and control (n=9). Animals received DBCM, BDCM and methanol, respectively at a concentration of 117µg/Kg, once daily, by gavage, to a total of four administrations. Methanol was used as vehicle for DBCM and BDCM. Animals from control group only received water. Animals were sacrificed four weeks after the administrations. Liver mitochondria were isolated and the mitochondrial respiratory activity (RCR), membrane potential ($\Delta\Psi$), bioenergetic activity and oxidative stress were measured.

Results: During the experimental protocol, 12 animals died and were excluded from the study. RCR and $\Delta\Psi$ were lower in DBCM, BDCM and methanol-exposed animals than in control group ($p<0.05$). Concerning mitochondrial bioenergetic activity, succinate dehydrogenase and ATP synthase activity was lower in DBCM and BDCM-exposed groups. However, cytochrome c oxidase activity was only lower in DBCM-exposed group ($p<0.05$). Concerning oxidative stress, the activity of superoxide dismutase and catalase was increased by DBCM, BDCM and methanol. Glutathione-s-transferase activity was significantly increased in both DBCM and BDCM groups ($p<0.05$).

Discussion and Conclusions: Mitochondrial analysis showed that THMs reduced mitochondrial bioenergetic activity and increased oxidative stress in hepatic tissues providing a biopathological basis for the hepatotoxicity of these compounds.

Notes:

Poster Presentations ESVP/ECVP

P143 EXPERIMENTAL GLIOBLASTOMA IN FISHER RAT MODEL: TREATMENT WITH A BIOCOMPATIBLE SYSTEM AS CARRIER OF METHOTREXATE DRUG

Notes:

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Introduction: Anticancer drug delivery through Solid Lipid Nanoparticles (SLN) to the brain parenchyma for the treatment of glioblastoma may represent a valid strategy aiming to overcome the BBB. Aim of the present study is to report the *in vivo* effects of SLN loaded with methotrexate drug (SLN-MTX) in glioblastoma treatment in Fisher rat model.

Materials and Methods: SLN-MTX biodistribution was evaluated in 12 rats thirty minutes after an intravenous injection. 12 rats implanted with F98 glioma cells line in caudate nucleus area were used to study tumour growth. Animals were imaged in a high field (7T) MRI scanner at postoperative day 7, 9 and 11. The MRI protocol included a T_1-w SE sequence before and after administration of a contrast medium. At postoperative day 7 and 9, six animals were treated intravenously with SLN-MTX. At day 11, all rats were sacrificed and submitted to a complete necropsy to perform histological and immunohistochemical investigations.

Results: The biodistribution showed a minimal increase of drug concentration in the brain. Tumour volumes (day 7, 9, 11) were calculated by MRI scanner. The growth curves seem to indicate a slowdown of tumour growth in treated animals compared to controls rats, although not significant. Histological examination of the brains confirmed the presence of tumour masses characterised by a different mitotic index and variable degrees of apoptosis, necrosis, vascular proliferation and glial reaction.

Discussion (and/or Conclusions): Preliminary data suggest a potential therapeutic effect of SLN-MTX. Additional histopathological investigations are in progress to confirm this hypothesis.

P144 PILOT STUDY WITH A MURINE MODEL TO FURTHER DISSECT THE PATHOGENESIS OF JAPANESE ENCEPHALITIS

Notes:

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Introduction: *Japanese encephalitis virus* (JEV) is a neurotropic flavivirus transmitted by culex mosquitoes and responsible for life-threatening encephalitides in humans and animals in east and south Asia. To fully reveal the pathogenesis of Japanese Encephalitis (JE) in humans, reproducible and comparable animal models are needed. Here we describe the neuropathological findings of a pilot study to assess the validity of a mouse model using intraperitoneal infection.

Materials and Methods: Six 10-month-old female C57BL/6 mice were intraperitoneally inoculated with a single dose of JEV (JEV P3 strain); a further four received PBS (controls). Animals were sacrificed after 10 days, when clinical signs developed. Brains were fixed in formalin and paraffin wax embedded. A detailed histopathological examination was performed and immunohistology for the demonstration of viral antigen, astrocytes (GFAP), microglia (Iba1), iNOS and apoptotic cells (cleaved caspase-3) undertaken.

Results: Infected animals exhibited severe non-suppurative encephalitis, affecting cerebrum and thalamus and characterised by 1-3 cell layer thick lymphocyte rich perivascular cuffs and vigorous microglial and astrocyte proliferation/activation. Viral antigen was detected in both intact and cleaved caspase-3 positive, apoptotic neurons. Iba1-positive microglial cells exhibited strong iNOS co-expression.

Conclusions: Type and distribution of lesions in our model mimicked JE in humans and experimental non-human primate infection and were highly consistent. Neuronal death was predominantly via apoptosis, possibly due to both a direct viral effect and as a bystander effect, mediated by the activated infiltrating and resident immune cells, and particularly microglial cells. The mouse model represents a suitable tool for JE pathogenesis studies.

Poster Presentations ESVP/ECVP

P145 IDIOPATHIC PULMONARY HYPERTENSION IN A DOG: INVOLVEMENT OF CARDIAC AND RENAL MICROVASCULATURE

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Introduction: Idiopathic pulmonary hypertension is an uncommon disease characterised by high blood pressure in lung´s blood vessels. A case of a 6.5 year-old male Akita dog with history of repeated syncope, vomiting, diarrhoea and anorexia is reported. Clinical examination found ascites, pale mucous membranes, heart rate 180 bpm, intense right side systolic sounds (4-5/6), breathing difficulties and syncope.

Materials and Methods: Necropsy, standard histology.

Results: Necropsy revealed an enlarged heart, concentric hypertrophy of right ventricle with 1:1 left to right ventricle ratio, distention of pulmonary artery, and thickening of tricuspid valve. Microscopic lesions included endothelial cell detachment and swelling of cell cytoplasm in heart arteries, severe degeneration of smooth muscle cells characterised by vesiculated and swollen nucleus and vacuolated cytoplasm up to cell death with fibrinoid appearance, that were consistent in the pulmonary vasculature including elastic arteries, arterioles and capillaries. Lesions in kidney were remarkable and affected complete nephrons, including swelling of glomeruli, thickened Bowman capsules with hyaline appearance and atrophy up to complete loss of these structures. The tubular epithelium had granular appearance with mild vacuolar degeneration and some foci of epithelial regeneration.

Discussion: The diagnosis of idiopathic pulmonary hypertension is made by exclusion criteria; it should be ruled out heart disease on the left side, cardiac and arterial congenital anomalies, chronic interstitial lung or airway disease or the presence of *Dirofilaria immitis*, none of these conditions were present in the case reported here

Notes:

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INHAND 01: PROLIFERATIVE AND NON-PROLIFERATIVE LESIONS OF THE RAT AND MOUSE MALE REPRODUCTIVE SYSTEM

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This presentation is part of the INHAND initiative (International Harmonization of Nomenclature and Diagnostic criteria for lesions in rat and mouse), a joint initiative of the Societies of Toxicologic Pathology from Europe (ESTP), Great Britain (BSTP), Japan (JSTP) and North America (STP). In the presentation several examples of both proliferative and non-proliferative lesions of the male reproductive organs of rats and mice will be presented for interactive discussion. Diagnosis and background information has been published by several authors and can be found in the Journal of Toxicologic Pathology:

Dianne Creasy, Axel Bube, Eveline de Rijk, Hitoshi Kandori, Maki Kuwahara, Regis Masson, Thomas Nolte, Rachel Reams, Karen Regan, Sabine Rehm, Petrina Rogerson and Katharine Whitney (2012) *Toxicologic Pathology* **40**, 40S-121S

Abstracts for INHAND

INHAND 02: FEMALE REPRODUCTIVE SYSTEM

Ute Bach[1] and Justin D. Vidal[2]

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INHAND (International Harmonization of Nomenclature and Diagnostic Criteria for Lesions in Rats and Mice) is a global, collaborative effort across multiple professional societies to review, update, and harmonise nomenclature for use in nonclinical toxicology studies. The OWG (Organ Working Group) for the female reproductive system has recently received comments from the GESC (Global Editorial Steering Committee) and professional society membership and has completed the final draft. There were a number of challenges concerning nomenclature and description of the different terms within the female reproductive system, e.g. how to handle physiologic changes occurring during the oestrous cycle, terminology of age-related changes, and nomenclature for cystic changes in/around the ovary. Additionally, the coordination across the multiple organs within the female reproductive system required several discussions. Drs. Ute Bach and Justin Vidal will present select examples of proliferative and non-proliferative lesions to highlight some of the more challenging areas. Opportunity for audience voting will be available during the presentation and will be followed by discussion.

Membership of the Female Reproductive OWG: Darlene Dixon (Chair), Ron Herbert (GESC Liaison), Roger Alison, Ute Bach, Karyn Colman, Johannes H. Harleman, Richard Haworth, Anke Heuser, Eric van Esch, George Foley, Jerry Long, Michael Mirsky, Karen S. Regan, Justin D. Vidal, and Midori Yoshida.

Abstracts for INHAND

INHAND 03-1: ENDOCRINE SYSTEM:

NON-PROLIFERATIVE AND PROLIFERATIVE LESIONS OF THE ADRENAL GLAND IN RODENTS

Annamaria Brändli-Baiocco

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Roche Innovation Center Basel, Switzerland

The INHAND (International Harmonization of Nomenclature and Diagnostic Criteria for Lesions in Rats and Mice) nomenclature is a global project to develop nomenclature and diagnostic criteria for classifying proliferative and non-proliferative histopathology lesions in laboratory animal species.

The working group for the endocrine system is composed by expert toxicologic pathologists from the Societies of Toxicopathology of Europe (ESTP), Great Britain (BSTP), Japan (JSTP) and North America (STP). The INHAND nomenclature and diagnostic criteria of the different organs of the endocrine system were discussed and agreed between the members of the group. They are now in a final draft status and published as first draft by the author in the internet (http://goreni.org). The manuscripts will be submitted for GESC and then for membership review soon.

Non-proliferative and proliferative lesions of the endocrine system may occur as spontaneous or chemically induced findings. In this presentation, selected non-proliferative and proliferative changes of the adrenal gland of rat and mouse will be presented and discussed with the relative differential diagnosis. Audience is requested to vote. An open discussion and a feedback on the nomenclature and diagnostic criteria are encouraged.

Proposed Terminology of Non-Proliferative Lesions of the Adrenal Gland in Rodents:

Angiectasis
Amyloidosis
Atrophy, cortical
Cyst (s)
Degeneration, cystic
Fibrosis
Ectopic tissue, adrenocortical
Hematopoiesis, extramedullary
Haemorrhage
Hypertrophy, cortical diffuse
Infiltrate, inflammatory cell
Inflammation
Mineralisation
Necrosis
Osseous metaplasia
Pigmentation
Thrombosis
Vacuolation cortical, increased, focal
Vacuolation cortical, decreased, focal
Vacuolation cortical, increased, diffuse
Vacuolation cortical, decreased, diffuse
X-zone persistence

Proposed Terminology of Proliferative Lesions of the Adrenal Gland in Rodents:

Hyperplasia, cortical
Adenoma, cortical
Carcinoma, cortical
Hyperplasia, subcapsular cell
Adenoma, subcapsular cell
Carcinoma, subcapsular cell
Hyperplasia, cortical
Hyperplasia, medullary
Pheochromocytoma, benign
Pheochromocytoma, complex, benign
Ganglioneuroma, benign
Pheochromocytoma, malignant
Pheochromocytoma, complex, malignant
Neuroblastoma, malignant
Myelolipoma

Members of the Endocrine Organ Working Group are:

Thomas Rosol (chair), Annamaria Brändli-Baiocco (Roche), Emmanuelle Balme (Boehringer Ingelheim), Marc Bruder (Novartis), Sundeep Chandra (GlaxoSmithKline), Jürgen Hellmann (Merck Serono Research), Mark Hoenerhoff (University of Michigan), Barbara Lenz (Roche), Mark Mense (Covance), Takahito Kambara, (GlaxoSmithKline), Susanne Rittinghausen (Fraunhofer ITEM), Hiroshi Satoh (Iwate University), Frédéric Schorsch (Bayer Cropscience), Frank Seelinger (AstraZeneca), Minoru Tsuchitani (Mitsubishi Chemical Medience), Zbigniew Wojinski (Drug Development Preclinical Services)

Reference:

Mann PC1, Vahle J, Keenan CM, Baker JF, Bradley AE, Goodman DG, Harada T, Herbert R, Kaufmann W, Kellner R, Nolte T, Rittinghausen S, Tanaka T (2012) International harmonization of toxicologic pathology nomenclature: an overview and review of basic principles. Toxicol Pathol. 2012, 40(4 Suppl): 7S-13S

Abstracts for INHAND

INHAND 03-2: ENDOCRINE SYSTEM:

PROLIFERATIVE LESIONS OF THE PITUITARY GLAND AND PINEAL GLAND IN RODENTS

Susanne Rittinghausen

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The INHAND project (International Harmonization of Nomenclature and Diagnostic Criteria for lesions in Rats and Mice) is a joint initiative of the Societies of Toxicologic Pathology from Europe (ESTP), Great Britain (BSTP), Japan (JSTP) and North America (STP) to develop an internationally-accepted nomenclature for proliferative and non-proliferative lesions in rodents used in regulatory as well as research studies. The Endocrine Organ Working is chaired by Thomas Rosol with members from all societies.

Proliferative lesions of the pituitary have a high spontaneous occurrence in male and female rodents, whereas proliferative lesions of the pineal gland are rare events.

In this interactive session, several of the proliferative lesions listed below will be shown and discussed with consideration of related differential diagnoses. The presented cases are going to be from rat, mouse or Syrian hamster and everybody in the audience will be asked to provide her/his diagnosis.

Proposed Terminology of Proliferative Lesions of the Pituitary Gland in Rodents

Carcinoma, pars distalis
Carcinoma, pars intermedia
Craniopharyngioma, malignant
Adenoma, pars distalis
Adenoma, pars intermedia
Craniopharyngioma, benign
Ganglioneuroma, benign
Pituicytoma
Hyperplasia, pars distalis
Hyperplasia, pars intermedia

Proposed Terminology of Proliferative Lesions of the Pineal Gland in Rodents

Pinealoma, malignant
Pinealoma, benign

Members of the Endocrine Organ Working Group:

Thomas Rosol (chair), Annamaria Brändli-Baiocco (Roche), Emmanuelle Balme (Boehringer Ingelheim), Marc Bruder (Novartis), Sundeep Chandra (GlaxoSmithKline), Jürgen Hellmann (Merck Serono Research), Mark Hoenerhoff (University of Michigan), Barbara Lenz (Roche), Mark Mense (Covance), Takahito Kambara, (GlaxoSmithKline), Susanne Rittinghausen (Fraunhofer ITEM), Hiroshi Satoh (Iwate University), Frédéric Schorsch (Bayer Cropscience), Frank Seelinger (AstraZeneca), Minoru Tsuchitani (Mitsubishi Chemical Medience), Zbigniew Wojinski (Drug Development Preclinical Services)

Reference:

Mann PC1, Vahle J, Keenan CM, Baker JF, Bradley AE, Goodman DG, Harada T, Herbert R, Kaufmann W, Kellner R, Nolte T, Rittinghausen S, Tanaka T (2012) International harmonization of toxicologic pathology nomenclature: an overview and review of basic principles. Toxicol Pathol. 2012, 40(4 Suppl):7S-13S

Abstracts for INHAND

INHAND 03-3: ENDOCRINE SYSTEM:

PROLIFERATIVE LESIONS OF THE THYROID GLAND AND PARATHYROID GLAND IN RODENTS

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The purpose of this work is to present and illustrate some of the proliferative lesions of the thyroid and parathyroid glands in rodents. Key differential diagnostic features are specifically highlighted.

Spontaneous thyroid follicular tumours are rare in rats and mice, and the majority is benign. The overall frequency in all mouse strains is approximately 1%. Proliferative thyroid follicular lesions are more common in rats than in mice, and males are more prone to developing such lesions than females in response to chronic TSH stimulation. The overall incidence in rats is less than 3%.

Follicular cell hyperplasia may be (multi)focal or diffuse. Glands affected by diffuse hyperplasia are typically enlarged bilaterally. Focal hyperplasia may arise in preexisting diffuse hyperplasia, and these areas of focal hyperplasia are occasionally compressive and not encapsulated. Follicles may be variable in size, with maintenance of normal follicular architecture. In rodents, progression from focal or diffuse hyperplasia to adenoma is common. Diffuse hyperplasia is characterised by an increase in the number of follicular epithelial cells throughout both glands, often in a microfollicular pattern composed of glands lined by cuboidal epithelial cells that may show papillary infoldings or pilling into glandular lumens. Atypia is not present.

Follicular adenomas are expansile, circumscribed and compressive proliferations of well-differentiated follicular epithelial cells arranged in branching papillary projections, dilated cystic proliferations, or solid sheets of variably sized follicles lined by one or multiple layers of well-differentiated epithelium. A capsule (complete or partial) may be present. Follicular or solid patterns composed of small follicles are termed microfollicular, whereas those with large or irregular follicles are macrofollicular. Mitoses are uncommon. Adenomas may be solitary, multiple, unilateral or bilateral. The differentiation between hyperplasia and adenoma is not well-defined: size is not a reliable criterion. Endocrinologically active follicular adenomas result in the colloid involution of adjacent follicles due to inhibition of TSH secretion.

Follicular carcinomas may be well differentiated and difficult to differentiate from adenoma. Carcinomas tend to have a more variable or heterogeneous cellular growth and may show evidence of cellular atypia and invasion of vascular, lymphatic, or adjacent thyroid gland and associated tissues. Solid carcinomas may be difficult to differentiate from C-cell neoplasm but tend to have a denser cytoplasm and better-defined cell borders. Carcinomas may be encapsulated and show evidence of capsular invasion. These neoplasms tend to be highly vascularised. A desmoplastic reaction, necrosis, mineralisation, pigment deposits and cholesterol cleft formation may be present. The mitotic rate is variable and distant metastases, predominantly to regional lymph nodes and lung, are uncommon. The incidence of benign and malignant tumours markedly increases when animals are exposed to chemicals that alter thyroid hormone homeostasis.

Focal and diffuse C-cell hyperplasia and adenomas are fairly common lesions in aged rats. Conversely, C-cell proliferative lesions are uncommon in the mouse. **C-cell hyperplasia** may be focal, multifocal, or diffuse. In rats, focal hyperplasia may occur concurrently with diffuse hyperplasia. Focal hyperplastic lesions may be difficult to differentiate from small C-cell adenomas and are composed of not encapsulated clusters of well-differentiated polygonal to round cells, with indistinct cell borders, abundant pale cytoplasm and central round nuclei. Diffuse C-cell hyperplasia is characterised by an increase in histologically normal C-cells distributed relatively uniformly between follicles. Atypia is generally not present in hyperplasia but may be observed in adenomas. When associated with hypercalcaemia or hyperparathyroidism, C-cell hyperplasia is typically diffuse in nature. An increase in proliferative C-cell lesions is seen in animals on a high-protein diet.

C-cell adenomas are very common in aged F344 rats, especially in males. Grossly, these tumours are typically whitish in color with a firm consistency (conversely, follicular tumours are red to brown in color and soft). As with follicular adenomas, C-cell adenomas can be single, multiple, unilateral, or bilateral and often cause compression of adjacent parenchyma, but do not invade. Neoplastic cells are arranged in clusters or sheets, with scant stroma, and are typically well-differentiated. The distinction between focal hyperplasia and adenoma has been designated by size; those lesions more than five follicles in diameter are considered adenomas. In addition, features of encapsulation, demarcation, compression and atypia should also be taken into consideration when differentiating large focal hyperplasias from smaller adenomas. The presence of amyloid, diagnostic in human tumours, is uncommon in rodents (more frequently seen in mice).

Spontaneous **C-cell carcinomas** are reported in rats at a low incidence and very rarely in mice. C-cell carcinomas are firm in consistency, whitish and poorly vascularised. Similar to adenomas, C-cell carcinomas may be unilateral or bilateral. Cells are arranged in sheets and nests separated by a fine fibrovascular stroma (with possible amyloid deposits) and may appear well differentiated and indistinguishable from adenomas, or pleomorphic with frequent mitoses. Poorly differentiated tumours may have a spindled appearance and exhibit weak immunoreactivity for calcitonin. Most C-cell carcinoma cells and some C-cell adenoma cells express somatostatin in addition to calcitonin. Local invasion or distant metastases to regional lymph nodes and lungs may be observed. Chemically induced C-cell tumours are very rare.

Parathyroid hyperplasia is usually due to disorders of calcium regulation. Hyperplasia is common with increasing age in F344 rats, usually as a result of chronic progressive nephropathy, particularly in males. Focal hyperplasia, less common than diffuse, may be unilateral or bilateral, may arise within preexisting diffuse hyperplasia, and is usually nonfunctional. Focal hyperplastic lesions may be minimally compressive or merge imperceptibly with surrounding normal tissue. Large focal hyperplastic lesions may be difficult to differentiate from small adenomas. Conversely, bilateral, diffuse chief cell hyperplasia and hypertrophy, secondary to nephropathy, are the most common parathyroid lesions in the F344 rat and very common in some strains of mice, where they are most often associated with renal amyloidosis. Administration of chemicals that interfere with calcium homeostasis can result in chronic stimulation of chief cells and diffuse hyperplasia. Hyperplasia due to dysregulation of calcium homeostasis or renal disease is bilateral and diffuse and is not associated with progression to neoplasia. With diffuse hyperplasia, there is global enlargement of the gland. Chief cells are packed together closely, often with indistinct cell boundaries. The expanded cytoplasmic area of chronically stimulated chief cells is lightly eosinophilic with occasional vacuoles.

Parathyroid gland tumours occur with low incidence in rats and mice. **Parathyroid adenomas** in rats are red to gray in color, encapsulated and solitary, causing enlargement of a single lobe. They are typically well circumscribed and compressive, composed of dense sheets and packets of well-differentiated cells separated by a fine fibrovascular stroma. Larger adenomas often nearly incorporate the entire affected gland. The cellular pattern may be very similar to hyperplastic lesions (solid growth) or may be papillary, cystic or acinar. Pleomorphism and mitotic rate are generally low but may be variable. Multiple white, pinpoint foci may be observed in thyroid glands in animals with functional parathyroid adenomas; these are areas of C-cell hyperplasia responding to chronic hypercalcaemia. Adenomas are usually non-functional in rats. The unaffected parathyroid glands may be atrophic if the adenoma is functional, normal if the tumor is non-functional, or enlarged by concomitant hyperplasia.

Parathyroid carcinomas are rare in rodents. Cells are usually arranged in solid sheets and may be pleomorphic. There is usually evidence of invasion through the parathyroid capsule. Extension to vessels or adjacent structures may be present, as well as metastases.

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Chandra S., Hoenerhoff M.J. and Peterson R. (2013). Endocrine glands. In *Toxicologic pathology – Nonclinical safety assessment* (Sahota P.S., Popp J.A., Hardisty J.F. and Gopinath C., eds.), pp. 655-716. CRC Press, Boca Raton, FL.

Speaker Abstracts

S01: A PRIMER OF COMPARATIVE THYROIDOLOGY: RELEVANCE TO ANIMAL MODELS OF THYROID DISRUPTION

Duncan C. Ferguson, VMD, PhD

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This presentation will begin by reviewing comparative aspects of thyroid hormone physiology, focusing on key differences between rodents, dogs, cats, subhuman primates and man. Characterisation of thyroid disruption require understanding key cellular targets in the hypothalamic-pituitary-thyroid axis, as well as the impact on serum thyroid hormone binding, deiodinative, and conjugative metabolism in peripheral tissues, and ultimately at the level of cellular thyroid hormone transporters and receptors.

As the understanding of thyroid hormone disruption by toxicants becomes more refined to cellular levels, it has become apparent that circulating total thyroid hormone (T_3 and T_4) concentrations provide at best a crude, and at worst, a misleading assessment of the animals true thyroid status, and may be completely irrelevant to understanding cellular thyroid perturbations, particularly during embryonic development. The central nervous system is particularly sensitive to both the deficiency and excess of thyroid hormone development, depending upon the stage of development.

Six key principles of the diagnosis of thyroid dysfunction used commonly by physicians and veterinarians will be outlined. The same principles should be employed when interpreting serum hormone concentrations in experimental animals.

1. With regards to immunoassays for iodothyronines (total T_3 and T_4), species-specific serum binding differences result in differences in detection vs. a standard curve prepared in no serum or serum from another species.

2. Accurate measurement of a low free T_4 and elevated TSH provide high diagnostic accuracy of the clinical state of primary hypothyroidism in most cases, with the following exceptions: a) Developing or recovering non-thyroidal illness; b) Xenobiotics which interfere with serum and possibly cellular binding of hormone

3. TSH is a specific-specific peptide hormone. Confirmation of cross-reactivity in a species-heterologous assay requires pure TSH standard from the species of interest, or demonstration of parallelism.

4. Current animal TSH assays are generally not sensitive enough to distinguish normal from low values, and therefore, they cannot confirm pituitary suppression and/or the diagnosis of hyperthyroidism.

5. Even considering allometric scaling, the free hormone concentration in serum governs cellular thyroid hormone delivery, metabolism and action in most species evaluated to date. Free T_4 should ideally be measured by equilibrium dialysis or an assay validated against this "gold" standard. Non-dialysis assays often simply correlate with total hormone measurements.

6. Total and free serum T_3 concentrations have shown to have very poor correlation to true thyroid status in an animal due to effects of non-thyroidal Illinois on thyroid hormone metabolism.

Finally, when pathologists observe thyroid follicular changes, when possible, interpretation should be made in the context of total and free T_4, and TSH concentrations.

Although not shown in the table below, the hyperthyroid state should be associated with elevated free T_4 (accurately measured) and decreased or, more likely, undetectable. Elevated T_4 and free T_4 concentrations have been associated with toxicants interfering with pituitary negative feedback of thyroid hormones (e.g. bisphenol as a TR-beta antagonist in the pituitary).

Species- Specific Thyroid Hormonal Changes Associated with Thyroid Follicular Changes Associated with Toxicological Pathology

Thyroid Follicular Pathology	Decreased Total T_4	Decreased Total T_3	Decreased Free T_4	Increased TSH
Decreased Follicle Size	R,M,D,P	R,M,D,P	R,M,D,P	R,M,D,P
Increased Follicular Height (Hypertrophy)	R,M,D,P	R,M,D,P	R,M,D,P	R,M,D,P
Decreased Colloid Area	R,M,D,P	R,M,D,P	R,M,D,P	R,M,D,P
Diffuse Hyperplasia	R,M,D,P	R,M,D,P	R,M,D,P	R,M,D,P
Focal Hyperplasia	R, (M)	R, (M)	R, (M)	R, (M)
Neoplasia	R, (M)	R, (M)	R, (M)	R, (M)

R = Rat; D = Dog; M = Monkey; P = Primate Parentheses indicate those effects that may be present at a lower incidence or degree. Developed with Dr. Thomas Rosol, Ohio State University

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Speaker Abstracts

S02: TESTING MIXTURES OF ENDOCRINE DISRUPTORS IN VIVO AT HUMAN-RELEVANT EXPOSURE LEVELS

Sibylle Gröters[1], Volker Strauss[1], Steffen Schneider[1], Karma Fussell[1], Stephanie Melching-Kollmuss[1], Benazir Siddeek[2], Mohamed Benahmed[2], Bennard van Ravenzwaay[1]

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Endocrine disruption has become an important topic of public concern. Despite an increasing amount of attention, little is understood about whether environmentally relevant doses of endocrine disrupting chemicals (EDCs) affect homeostasis. Furthermore, knowledge gaps often exist in the studies used to assess EDCs. To address these concerns we performed pre-/post-natal reproductive toxicity studies to measure the developmental toxicity of low single- and mixture-doses of three anti-androgens. During these studies a number of endpoints including weight assessment of reproductive organs and histopathology was performed.

Doses were selected to mimic a low observed adverse effect level (LOAEL), the no observed adverse effect level (NOAEL) for endocrine effects, and the acceptable daily intake (ADI) for each compound, which were then additionally combined together into three mixtures of the LOAELs, NOAELs, and ADIs. The endocrine consequences of a larger effect dose of flutamide were also evaluated as a positive control.

While female offspring developed normally, the male offspring demonstrated anti-androgenic effects at the single and mixed LOAEL doses only. A significant decrease in anogenital distance on PND 1 and an increase in the number of nipples/areolas on PND 12 were noted in these animals. The latter effect was partially transient, as all had regressed by PND 20, except at the positive control dose. The male offspring in these dose groups which were reared to young adulthood displayed additional anti-androgen effects including delayed sexual maturation and reduced sex organ sizes and weights; offspring from the positive control dose also had an increased incidence of developmental sexual defects. No adverse effects were noted at either the NOAEL or ADI dose levels. Assessment of additional endpoints including the transcriptome, miRNome and metabolome (with hormone levels) as well as histopathological phenotypical anchoring is ongoing.

Speaker Abstracts

S03: ENDOCRINE REGULATION AND ENDOCRINE MEDIATED DISTURBANCES IN THE ADULT MALE REPRODUCTIVE SYSTEM

THE BSTP Chirukandath Gopinath Lecture

Dianne M Creasy

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The morphology and function of the adult male reproductive tract is regulated by endocrine as well as by local paracrine hormonal control. The hypothalamic-pituitary-gonadal pathway involves a complex interplay between GnRH from the hypothalamus, FSH, LH, and prolactin from the pituitary and testosterone plus its metabolites from the testis and reproductive tract. The role of these hormones in regulating reproductive function varies significantly between species, making it essential for the toxicologic pathologist to be aware of the comparative endocrinology of rodents, canines and primates when evaluating nonclinical pharmaceutical programs. The same hormone disturbance in different species can produce dramatically different morphologic responses and functional outcome.

Whatever the site of hormonal disturbance, the morphological changes seen by the pathologist, in most cases, reflect altered production or metabolism of testosterone or altered sensitivity of androgen receptors to local or circulating androgens. All of the tissues in the male reproductive tract (pituitary, testis, epididymis, and accessory sex organs, plus mammary gland in rats) are affected by hormonal status, some more than others, but different profiles of change will occur, depending on the nature of the hormonal disturbance. For example androgen receptor antagonism generally has no detectable effects on spermatogenesis, but causes diffuse Leydig cell hyperplasia, and marked atrophy of the epididymis and accessory sex organs. This contrasts with the effects caused by inhibition of testosterone steroidogenesis, which causes stage specific germ cell death, progressive loss of elongating spermatids, atrophy of the Leydig cells and moderate atrophy of the accessory sex organs. Both types of homone disturbance will result in increased incidences of Leydig cell tumours with prolonged dosing. Understanding the different profiles of change and what to look for in the different tissues allows the toxicologic pathologist to gain insight into the likely mechanism of hormonal disturbance, and design appropriate investigative studies. Understanding the underlying endocrine pathways is also essential for explaining the changes.

Leydig cell tumours are a common response to chronic imbalance of reproductive hormones in rodents but the mechanism is different between rats and mice. Although the induction of Leydig cell tumours in rodents is considered to have limited relevance for human risk, it is very helpful for regulatory purposes to conduct some basic investigative studies to provide a mode of action to explain which of the various endocrine pathways have been affected. Measurement of reproductive hormones is an obvious and attractive option for investigating hormonal disturbances, but their pulsatile secretion, sensitivity to stress and general inter- animal variability require that appropriate numbers of animals per group or adequate numbers of samples per animal are collected, to provide any chance of detecting meaningful results. For measuring testosterone in rats, this often means using 20-30 rats/group and for dogs and primates, a multiple sampling regime and multiple time point evaluations are recommended, as well as using each animal as its own internal control. Duration of dosing and time after dosing are also important factors to consider when designing hormonal studies, otherwise short lived changes in hormone levels can be missed or adaptive changes in hormone receptor sensitivity can normalize circulating hormone levels following prolonged dosing. Measurement of circulating hormones requires carefully designed studies with adequate numbers of animals/samples and suitable statistical analysis.

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Part 1 Introduction: http://www.oecd.org/chemicalsafety/testing/40638492.pdf

Part 2: Male reproductive system. http://www.oecd.org/chemicalsafety/testing/40580640.pdf
http://www.oecd.org/chemicalsafety/testing/40580693.pdf;
http://www.oecd.org/chemicalsafety/testing/40580720.pdf

Part 4: Mammary gland. http://www.oecd.org/chemicalsafety/testing/40613059.pdf
Part 6: Pituitary: http://www.oecd.org/chemicalsafety/testing/40581416.pdf.

Stanislaus D, Andersson H, Chapin R, Creasy D, Ferguson D, Gilbert M, Rosol TJ, Boyce RW, Wood CE 2012. Assessment of Circulating Hormones in Regulatory Toxicity Studies I General Concepts and Considerations. Toxicol Pathol 40, 943-950

Speaker Abstracts

S04: ENDOCRINE DISRUPTERS, MALE REPRODUCTIVE DISORDERS AND LOW FERTILITY RATES

Niels E. Skakkebaek

University of Growth and Reproduction, Rigshospitalet, Copenhagen, Denmark

During the past decades there has been a quite dramatic increase in testicular cancer, which is a disease of young men. In some countries like Denmark and Norway almost 1% of all men are now treated for this disease. Furthermore, prospective studies show that poor semen quality is common: 20-40% of young men in several European and Asian countries have suboptimal sperm counts and more than 90% of sperms from otherwise healthy men are classified as abnormal. In addition, we have over the past 50 years witnessed a sharp decline in fertility rates in most of the industrialised countries all over the World. Interestingly, Danish data have shown a strong correlation to year of birth with more recent birth cohorts having higher incidences of reproductive problems, including testis cancer and lower semen quality and testosterone levels.

A hypothesis has been put forward that testicular cancer, undescended testis, hypospadias, low testosterone levels and spermatogenic disorders may be linked through a testicular dysgenesis syndrome (TDS) of fetal origin (Juul et al.). Data indicating that precursor cells of testicular cancer are derived from primordial germ cells will be presented, including their embryonic phenotype with expression of OCT4, C-Kit and PLAP. The hypothesis suggests that both genetic and environmental factors, including endocrine disrupters, contribute to an increasing trend in male reproductive disorders. However, the convincing trend with several fold increase in number of men with the disease strongly suggest that environmental factors dominate in pathogenesis of testicular cancer.

Endocrine disrupters may interact at several other processes involved in germ cell development and fertilisation. In fact, an ongoing project suggests that some endocrine disrupters may also interfere directly with functions of mature sperms and thereby contribute to lower fertility (Schiffer et al.).

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Speaker Abstracts

S05: EFFECTS ON RAT REPRODUCTIVE SYSTEM AFTER DEVELOPMENTAL EXPOSURE TO MIXTURES OF ENDOCRINE DISRUPTING COMPOUNDS

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Several compounds in the environment have been shown to act as endocrine disrupters, including pesticides, plasticisers and cosmetic ingredients. It is well documented that developmental exposure to anti-androgens can adversely affect male reproductive development. More recently, rodent studies have indicated that also female reproductive development can be adversely affected by perinatal exposure to environmental endocrine disrupters, as effects on mammary development, ovarian folliculogenesis, fertility and onset of puberty and of menopause have been reported. These findings contribute to the increased awareness of the impact of endocrine disrupters in early development. Since little is still known on long lasting effects of endocrine disrupters, the aim of the present study was to investigate both early and late effects.

In a study on perinatal exposure of Wistar rats to a mixture of human relevant endocrine disrupting chemicals, the influence on the female and male reproductive systems were examined. The mixture consisted of 13 anti-androgenic or oestrugenic chemicals, including phthalates, pesticides, cosmetic ingredients, bisphenol A and paracetamol, and the mixture ratio reflected high-end human intakes. Throughout gestation and lactation time-mated rats (n=16-20 dams) were exposed to the total mixture (TotalMix) at 100, 150, 200 or 450 times high-end human intake, or to mixtures of only the anti-androgens (AAMix) or only the estrogens (EMix) at 200 or 450 times human intake.

Pathological changes were found for male and female offspring for the two mixtures including anti-androgens (AAmix and TotalMix groups). In males, ano-genital distance was decreased perinatally, and prepubertally nipple retention was increased and prostate weight was decreased. At 1-1.5 year of age, changes in reproductive organs were observed in the same exposure groups. In 19 months old male offspring, decreased sperm count and increased prevalence of hyperplasia in prostates were found. Signs of earlier reproductive senescence (menopause) and decreased ovary weights were seen in female offspring. Histological examination of one ovary section from each 1-year old female revealed an increased incidence of rats with complete lack of corpora lutea in the AAMix group.

These studies showed that developmental exposure of rats to endocrine disrupters induced early as well as long-lasting effects on male and female reproduction. Adverse reproductive effects were observed at mixtures reflecting 100-200 times high end human exposure. Thus, a safety margin of 100 usually required for regulatory purposes may not be obtained for highly exposed pregnant women, suggesting that highly exposed human population groups may not be sufficiently protected against endocrine disrupting effects of environmental chemicals.

Speaker Abstracts

S06: EVALUATION OF THE MAMMARY GLAND IN SAFETY STUDIES

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The mammary gland is a standard organ in the histopathological examination of preclinical safety studies. The development of this organ system and functionality is driven by hormonal regulations. It is therefore not surprising that the new OECD guideline on endocrine disruptors also include this organ.

In rats there is a marked sexual dimorphism between male and female animals especially in young adults. (Cardy, 1991). The glands in male animals have a lobuloalveolar structure, whilst in female animals there is a tubuloalveolar structure. This difference is especially pronounced in the inguinal area. The difference disappears upon aging, possibly due to the increased prolactin levels observed in aging rats. The morphology of the male and female mammary gland can give sensitive information on the presence of hormonal effects in a study (Lucas et al, 2007). Compounds which increase prolactin or GR agonists cause a feminisation of the male mammary gland and a lobular alveolar hyperplasia and secretion in the female mammary gland. Oestrogen receptor modulators cause an atrophy of the male mammary gland and a virilisation of the female morphology. An awareness of such response patterns is important for the toxicological pathologist in the recognition of potential endocrine changes and should be seen in conjunction with changes in the other endocrine organs.

Although the hormonal control mechanisms in rodents are similar to humans, there are also differences. Upon aging the prolactin levels in rodents are increasing. This is correlated with the development of hyperplasia and adenomas in the pituitary. The sustained increased levels of prolactin are correlated with the development of galactocoeles, mammary hyperplasia and mammary tumours. Compounds which stimulate prolactin secretion result in carcinogenicity studies in an increased incidence of mammary tumours. (Hargreaves and Harleman, 2011). On the other hand compounds or treatments, which reduce the secretion of prolactin have a lower incidence of mammary tumours in carcinogenicity studies. In such studies one can also observe an increased incidence of uterine adenocarcinomas. The latter is due to a rodent specific effect of prolactin on the ovary. Prolactin is luteotrophic in rats, but not in primates. The lower levels of prolactin result in a relative oestrogen dominance and prolonged cycling. Reduced prolactin levels and changes in tumour patterns with decreased incidence of pituitary and mammary tumours and increased incidence of uterine tumours have also been observed in food restriction studies resulting in reduced body weight gain. Hence this pattern of response of reduced mammary tumours and increased incidence of uterine tumours has no relevance for man. In chronic toxicity studies and carcinogenicity studies with dose levels above the MTD resulting in a reduced body weight gain one can also observe a similar pattern of change in tumours incidence (Harleman et al, 2012). An analysis of the RITA database for the co-incidence of uterine and mammary tumours showed that animals with a mammary tumour were less likely to have an uterine tumour, confirming the presence of these interrelations of tumour incidences also in control animals. This effect was stronger in Wistar rats than in Sprague Dawley animals.

In the dog an awareness of mammary morphology is important in the evaluation of juvenile status and oestrus cycle. In this non-rodent species frequently young adult animals are used. Depending upon the age, the animals are often just before the first heat upon entry of the study. Because of the prolonged cycle in this species, the mammary gland morphology provides an excellent opportunity to judge the oestrus stage and sexual maturation of the animal (Harleman and Foley, 2001)

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Speaker Abstracts

S07: PITUITARY GLAND

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The pituitary gland (hypophysis) is divided into two major compartments: (1) the adenohypophysis (anterior lobe) composed of the *pars distalis*, *pars tuberalis*, and *pars intermedia*; and (2) the neurohypophysis (posterior lobe). In most animals and in human fetuses, the thin cellular zone between the adenohypophysis and neurohypophysis is referred to as the *pars intermedia* or intermediate lobe.

The pituitary lies within the *sella turcica* of the sphenoid bone. The gland receives its blood supply via the posterior and anterior hypophyseal arteries, which originate from the internal carotid arteries. Arteriolar branches penetrate the pituitary stalk and form a capillary plexus near the median eminence. These vessels drain into the hypophyseal portal veins, which supply the adenohypophysis. This hypothalamic-hypophyseal portal system transports hypothalamic-releasing and release-inhibiting hormones directly to the adenohypophysis for interactions with their specific target cells.

The adenohypophysis represents the largest portion of the pituitary gland. The cells within this lobe are responsible for the synthesis of at least six major hormones: growth hormone (GH), prolactin (PRL), and adrenocorticotropin (ACTH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), and thyroid-stimulating hormone (TSH, thyrotropin). With H&E staining, cells of the anterior lobe have been divided into acidophils (GH, PRL), basophils (LH, FSH, TSH), and chromophobes (ACTH and other types), constituting approximately 40, 10, and 50% of the total cell population, respectively.

In addition to specific hormone-secreting cells, a population of supporting cells is also present in the adenohypophysis. These cells have been referred to as stellate (follicular) cells and can be stained selectively with antibodies to S-100 protein. Stellate cells typically have elongate processes and prominent cytoplasmic filaments. These cells appear to provide a phagocytic or supportive function in addition to producing a colloid-like material.

The hypothalamus serves as the major regulator of the adenohypophysis. Each cell type within the adenohypophysis is under the control of a corresponding releasing hormone that is synthesised within nerve cell bodies of the hypothalamus. The releasing hormones are transported via axonal processes to the median eminence where they are released into capillaries and are carried by the hypophyseal portal system to trophic hormone-producing cells in the adenohypophysis. Specific releasing factors have been identified for TSH, FSH and LH, ACTH, and GH. Prolactin (PRL) secretion is stimulated by a number of factors, the most important of which appears to be thyrotropin-releasing hormone (TRH). TRH stimulates the release of prolactin with many of the same dose-response characteristics as the stimulation of TSH release.

Multiple influences contribute to the control of adenohypophyseal hormone secretion. Dopamine serves as the major prolactin inhibitory factor. Dopamine also suppresses ACTH production by corticotrophs in the *pars intermedia* of some species. A second hypothalamic release inhibiting hormone is somatostatin (somatotropin release-inhibiting hormone, SRIH). This tetradecapeptide inhibits the secretion of both growth hormone and TSH. In some situations, SRIH also inhibits the secretion of PRL and ACTH. The control of pituitary hormone secretion is also affected by negative feedback loops resulting from the interaction of end organ hormones, adenohypophyseal hormones, and corresponding hypothalamic releasing-and release-inhibiting hormones.

Pituitary tumours can be induced by sustained uncompensated hormonal derangements leading to increased synthesis and secretion of pituitary hormones. In such a situation, the absence of feedback inhibition of the pituitary cell may lead to its unrestrained proliferation. This effect can be potentiated by the concurrent administration of ionising radiation or chemical carcinogens.

For example, in the case of the pituitary-thyroid axis, T_4 and T_3 normally control the pituitary secretion of TSH in a classical negative feedback loop. Surgical removal or radiation- induced ablation of the thyroid or interference with the production of thyroid hormones by the use of specific inhibitors leads to the prolonged stimulation of TSH synthesis and secretion. As an initial response, TSH synthesis is enhanced markedly and is reflected by an increase in polyribosomes and granular endoplasmic reticulum within the cells. Typically, cisternae of the granular endoplasmic reticulum become dilated and filled with a flocculent proteinaceous material. There is a concurrent increase in the number of saccules and vesicles of the Golgi apparatus. These cells appear hypertrophic at the light microscopic level.

Hyperplasia of the thyrotrophs occurs concurrently with cellular hypertrophy as a consequence of the absence of normal inhibitory feedback mechanisms. Foci of hyperplasia may progress to the formation of adenomas. The role of gonadectomy in pituitary tumor induction has been studied most intensively in mice. Pituitary tumours induced by gonadectomy in this species are markedly strain-dependent and may contain FSH, LH, or both.

The administration of oestrogens is a reproducible method for inducing pituitary tumours in some experimental animals. The effect of exogenous oestrogen on the rat pituitary includes stimulation of prolactin secretion and the induction of prolactin-secreting tumours. The administration of oestrogens in susceptible strains results in increased serum prolactin levels, increased numbers of prolactin cells within the pituitary, and increased mitotic activity. There is considerable variation in the inducibility of pituitary tumours by oestrogens in different rat strains. For example, F344 rats are more sensitive than Holtzman rats.

The precise mechanisms of estrogen-induced prolactin tumours are unknown. Such tumours have been associated with a loss of hypothalamic dopaminergic neurons, which normally inhibit the function of prolactin-secreting cells. When transplanted subcutaneously, prolactin-producing tumours were also associated with degenerative changes in hypothalamic dopaminergic neurons, suggesting that prolactin itself could be neurotoxic.

The tumorigenic action of oestrogen may not be due exclusively to its effect on the hypothalamus because oestrogen can produce prolactinomas in pituitaries grafted beneath the renal capsule. Dopamine agonists, including lisuride and bromocriptine, antagonise the direct stimulatory effects of oestrogens on the prolactin cells. Dopamine agonists may act directly on dopaminergic receptors within the transplanted pituitaries. Prophylactic treatment of rats with lisuride decreases the incidence of pituitary adenomas in old animals. The mean levels of prolactin in the serum are considerably lower in lisuride-treated rats than in controls.

Other agents, including caffeine, have also been implicated in the development of pituitary adenomas. Additionally, the administration of N-methylnitrosourea is also associated with the development of pituitary adenomas in Wistar rats. The neuroleptic agent sulpiride has been reported to cause the release of prolactin from the anterior pituitary in the rat and to stimulate DNA replication. The administration of clomiphene prevents the stimulation of DNA synthesis produced by sulpiride, but does not affect prolactin release from the gland. These findings have suggested that the intracellular prolactin content of the anterior pituitary plays a role in the regulation of DNA synthesis through a mechanism mediated by oestrogens.

Administration of salmon calcitonin for 1 year to Sprague-Dawley and Fisher 344 rats was associated with an increased incidence of pituitary gland hyperplasia and adenomas. The pituitary tumours produced the common alpha subunit of the glycoprotein hormones (LH, FSH, and TSH), a type of tumours that has been shown to comprise a significant fraction of pituitary tumours in humans. After treatment with calcitonin, serum alpha-subunit levels were increased in male Sprague-Dawley and Fischer rats. There was a good correlation between histopathologic evidence of alpha-subunit-producing pituitary tumours and elevated serum levels.

Studies have not determined whether the effects of calcitonin are direct or indirect. Calcitonin is produced in large amounts in the posterior hypothalamus and median eminence where it may normally exert an effect on the hypothalamus-pituitary axis. Calcitonin receptors have been identified in the hypothalamus, and lower numbers of receptors are found in the pituitary gland. A striking feature of calcitonin-induced pituitary tumours and elevated serum alpha-subunit levels was the predilection for male compared with female rats. The basis for sex- and species-specific effects of calcitonin has not been determined. The relevance of the effects of calcitonin in the rat pituitary gland to human pathophysiology is uncertain. Neither the treatment of patients with calcitonin nor patients with the multiple endocrine neoplasia syndrome II with medullary thyroid cancer and elevated calcitonin levels have resulted in the development of pituitary tumours; however, treatment with calcitonin may increase the overall incidence of cancer. The doses of calcitonin used were from 25- to 50-fold greater on a per weight basis than doses administered to patients. In addition, rats of several strains are known to be highly predisposed to develop pituitary tumours compared to humans.

Numerous hypotheses have been invoked to explain the high incidence of pituitary adenomas in certain inbred rat strains. Both hereditary factors and the levels of circulating sex steroids have been suggested as important etiological mechanisms. The hypothalamus has also been incriminated in the development of these tumours. Age-related hypothalamic changes may result in the diminished activity of dopamine, the major prolactin inhibitory factor. In this regard, studies of aging male rats have demonstrated abnormal concentrations and metabolism of hypothalamic dopamine, norepinephrine, and serotonin and decreased hypothalamic prolactin inhibitory activities.

Humans have a high incidence of pituitary adenomas (20-25% of the population in autopsy or MRI studies) and approximately 1/3 are associated with clinical signs. The incidence of undiagnosed adenomas is 30% in people between 50-60 years of age. Most tumours occur in the pars distalis and include adenomas of corticotrophs (10-15%), somatotrophs (10-15%), lactotrophs (35%), somatolactotrophs (5%), thyrotrophs (2%), and gonadotrophs (35%).

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Speaker Abstracts

So8: ADRENAL GLAND

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The adrenal cortex is the most common target organ for toxicity in the endocrine system and factors contributing to this vulnerability have been recognised. First, there is the dependence of the adrenal cortex on the trophic support of hormones from the pituitary and hypothalamus and also hormones from other endocrine tissues. Additionally, the adrenal cortex has both anatomic and molecular characteristics that convey vulnerability to toxic insult and the following factors predispose the adrenal cortex to toxic insult.

- Functional dependence on the hypothalamus and pituitary and peripheral hormone-carrier molecules (e.g., cortisol binding globulin, CBG).
- The large number of potential toxicological targets such as enzymes, receptors, and biochemical functional mediators.
- The sequentially dependent steroidogenic steps in cortisol/corticosterone or aldosterone production and secretion are at the end of the pathway with highest probability of effect from upstream toxicity.
- High vascularity and large blood volume received per unit mass of adrenal tissue ensuring exposures to toxicants. However, the adrenal lacks a significant direct arterial blood supply to the deep cortex. The deep zones receive blood containing reduced oxygen and previously secreted steroids potentially influencing CYP induction profiles.
- The high content of unsaturated fatty acids in adrenocortical cell membranes that are susceptible to lipid peroxidation both directly and indirectly.
- Lipophilicity due to rich cholesterol and steroid content favoring deposition of lipophilic compounds.
- The high content of CYP enzymes present in the adrenal cortex that can produce reactive metabolites of toxicants that then mediate toxicity, hydroxylation reactions, and generate free radicals.

Classes of chemicals known to be toxic for the adrenal cortex include short chain (three or four carbon) aliphatic compounds, lipidosis inducers, and amphiphilic compounds. It would also appear that hormones, especially exogenous steroids, have a direct effect on the adrenal cortex. A variety of other compounds may also affect the cortex. The most potent aliphatic compounds are of three-carbon length with electronegative groups at both ends. These compounds frequently produce necrosis, particularly in the *zonae fasciculata* and reticularis. Examples include acrylonitrile, 3-aminopropionitrile, 3-bromopropionitrile, l-butanethiol, and 1,4-butanedithiol. By comparison, lipidosis inducers can cause the accumulations (often coalescing) of neutral fats, which may be of sufficient quantity to cause a loss of organellar function and cellular destruction. The *zonae reticularis* and *fasciculata* appear to be the principal targets of xenobiotic chemicals. Examples of the compounds causing lipidosis include aminoglutethimide, amphenone, anilines and imidazole antimycotic drugs. Biologically active cationic amphiphilic compounds tend to produce a generalised phospholipidosis that involves primarily the *zonae reticularis* and *fasciculata*. They cause microscopic and subcellular phospholipid-rich inclusions. These compounds affect the functional integrity of lysosomes, which appear ultrastructurally to be enlarged and filled with membranous lamellae or myelin figures. Examples of compounds known to induce these types of effect include chloroquine, triparanol, and chlorphentermine.

Another class of compounds that affects the adrenal cortex is certain hormones, particularly natural and synthetic steroids. Some of these steroid hormones (corticosteroids) may cause functional inactivity and morphological atrophy during prolonged exogenous use. Other steroid hormones (natural and synthetic oestrogen and androgens) have been reported to cause proliferative lesions in the adrenal cortex of laboratory animals.

The final class of compounds represents a miscellaneous group of chemicals that affect hydroxylation and other functions of mitochondrial and microsomal fractions (smooth endoplasmic reticulum). Examples of these compounds include o,p'-DDD and α1-(l,4-dioxido-3-methylquinoxalin-2-yl)-N-methylnitrone (DMNM). Additional compounds in this category cause their effects by means of cytochrome P450 metabolism and the activation of toxic metabolites. An example is the activation of carbon tetrachloride, resulting in lipid peroxidation and covalent binding to cellular macromolecules of the adrenal cortex. Harvey et al (2009) review over 70 compounds and their targets in adrenocortical cells, including down regulation of the ACTH receptor or Steroid Acute Regulatory (StAR) protein, through to inhibition of steroidogenic enzymes. Of note is that compounds may have multiple targets and for example, aminoglutethimide down regulates the ACTH receptor, inhibits CYP11A1 (cholesterol side chain cleavage) and CYP11B1 (CYP11β/18), which is the terminal enzyme in cortisol synthesis.

Most chemicals affecting adrenal function appear to do so by altering steroidogenesis. A study of the effects of these chemicals on the histology and ultrastructure of adrenal cortical cells often can give insight into possible selection sites of inhibition of steroidogenesis. For example, chemicals causing increased lipid droplets may be involved in inhibiting the utilisation of steroid precursors, including the conversion of cholesterol to pregnenolone. Chemicals that affect the fine structure of mitochondria and smooth endoplasmic reticulum would be expected to impair the activity of 11α-hydroxylase and l7α- and 2l-hydroxylases, respectively. The previously mentioned examples of impaired steroidogenesis would result in lesions found primarily in the *zonae reticularis* and *fasciculata*. However, atrophy of the *zona glomerulosa* may reflect specific inhibition of aldosterone synthesis or secretion, either directly (inhibition of l8Q1-hydroxylation) or indirectly (inactivation of the renin-angiotensin system II), by chemicals such as spironolactone and captopril, respectively. The inhibition of steroidogenesis in some situations is nonspecific, as many hydroxylation reactions are affected, such as with carbon tetrachloride and cadmium intoxications.

Proliferative lesions of the medulla, particularly in the rat, have been reported to develop as a result of a variety of different mechanisms. For example, the long-term administration of growth hormone is associated with the development of pheochromocytomas. Prolactin-secreting pituitary tumours, which occur commonly in many rat strains, may play a role in the development of proliferative medullary lesions. Growth hormone and prolactin effects may be related because the two hormones are approximately 40% homologous; growth hormone, when present in high concentrations in the circulation, can bind to prolactin receptors. Oestrogen and thyrotropin-releasing hormone also would be expected to stimulate prolactin release. It is also possible that certain neuroleptics increase prolactin by decreasing dopamine release.

Both nicotine and reserpine have been implicated in the development of adrenal medullary proliferative lesions. Both agents may act by a shared mechanism, as nicotine directly stimulates nicotinic acetylcholine receptors, whereas reserpine causes a reflex increase in the activity of cholinergic nerve endings in the adrenal. Treatment with antithyroid drugs such as propylthiouracil also may affect chromaffin cells by a similar mechanism, as hypothyroid rats have increased sympathetic activity. An additional component of the action of reserpine occurs through the depletion of hypothalamic dopamine stores.

Several other drugs have been reported to increase the incidence of adrenal medullary proliferative lesions. These include zomepirac sodium (a nonsteroidal anti-inflammatory drug), isoretinoin (a retinoid), and gemfibrozil (a hypolipidemic drug). However, the mechanisms responsible for the stimulation of adrenal medullary proliferation by these drugs are unknown. Elmore et al (2009) provide a recent review of the adrenal medulla as a toxicological target organ, provide an up to date list of chemicals producing treatment-related pheochromocytoma and provide recent background control incidence of adrenomedullary tumours in in B6C3F1 mice and F344 rats, showing striking species and sex variation. There is an association with severe nephropathy and lung pathology (specific to inhalation studies where pulmonary fibrosis and inflammation results in hypoxaemia) with pheochromocytoma.

Nutritional factors have an important modulating effect on the spontaneous incidence of adrenal medullary proliferative lesions in rats. Several sugars and sugar alcohols have produced adrenal medullary tumours at high dosages, including xylitol, sorbitol, lacitol, and lactose at concentrations of 10 to 20% in the diet. Although the mechanism involved is not completely understood, a role for calcium (Ca2+) has been postulated. High doses of slowly absorbed sugars and starches increase the absorption and urinary excretion of Ca2+. Hypercalcaemia is known to increase catecholamine synthesis in response to stress, and low-calcium diets will reduce the incidence of adrenal medullary tumours in xylitol-treated rats. Other compounds that might act via altered Ca2+ homeostasis include the retinoids (which will produce hypercalcaemia) and conditions such as progressive nephrocalcinosis in aging male rats treated with nonsteroidal anti-inflammatory agents.

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Speaker Abstracts

S09: FEMALE REPRODUCTIVE SYSTEM
Part I: Endocrinology and Evaluation of the Female Reproductive System in General Toxicology Studies

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Evaluation of the female reproductive system in a general toxicology setting can be difficult and at times frustrating for the toxicologic pathologist. The cyclic nature of the oestrus and menstrual cycles drives the marked variability in the size, shape, and appearance of the reproductive organs in normal animals. The impact of puberty and reproductive senescence further complicates the picture. In addition, the reproductive strategies of our commonly used laboratory animals are wildly different. As result, the toxicologic pathologist is required to have a thorough understanding of the normal anatomy, physiology, and histology of each of these species prior to deciding what is abnormal. There are several key concepts that will enable the toxicologic pathologist to manage the inherent variability present in the female reproductive system.

1) The primary role of the toxicologic pathologist in a general toxicology setting it to evaluate end organ toxicity, NOT to detect alterations in cycle dynamics or subtle physiologic changes. For most indications, additional reproductive toxicology studies will be performed with larger sample sizes and longitudinal assessment of the cycle.
2) Always evaluate pituitary, ovary, uterus, cervix, vagina, and mammary gland together.
3) Animals will not be prescreened or synchronised at necropsy. It is typical to have a mix of normal cycle stages with a few abnormal animals at the end of the study.
4) In most cases it is NOT helpful to document cycle stage in the tables. You are not looking for numeric changes or prolongation in a given cycle stage, which would require a longitudinal assessment (daily vaginal cytology with baseline) and cannot effectively be done with a single time point measurement at necropsy. In fact, prolongation of a cycle stage is a misnomer based off of clinical observations. For example, repeated observations over several days of lordosis and keratinised cells using vaginal cytology leads to the clinical diagnosis of persistent oestrus (because clinically it looks just like oestrus); however, histologically the ovary is not characteristic of oestrus and lacks the expected new corpora lutea from recent ovulation. Depending on the cause there may be large follicular cysts in the case of reproductive senescence or atrophy due to an oestrugenic xenobiotic- either way, despite the oestrugenic stimulation present in both cases, the histologic picture is not one of normal oestrus and should not be diagnosed as such.
5) Use morphologic terms NOT cycle stages for diagnoses. The example of an oestrugenic xenobiotic above is the perfect example. The oestrugenic stimulation leads to ovarian atrophy and vaginal hyperplasia with keratinisation. Certainly the vagina has shared features of oestrus, but diagnosing "oestrus" for the vagina implies a normal stage of the cycle, which this animal is certainly not in and is not even cycling as the HPG axis is shut down due to the negative feedback from the oestrugenic xenobiotic.
6) Rat: The rat has a very rapid 4-5 day cycle and histologic changes, while very characteristic can display significant variability in the normally cycling rat. Cervical stimulation is needed for significant progesterone production and the corpus luteum of a cycling rat is practically incompetent compared to the activity following mating and as such the corpora lutea in a general toxicity study are quite different from those counted in a female fertility study. Reproductive senescence is well characterised aging change in rodents with distinct histologic changes and timing of onset can vary depending on strain and housing practices.

7) Dog: The bitch has a 100+ day reproductive cycle. In all likelihood, most animals will be in dioestrus or anoestrus at necropsy and many of them will not have even ovulated during the course of a study. As such, the dog is a poor model of effects on the HPG-axis or oestrus cycle, but can be sensitive to hormonal effects on the tubular tract.

8) Non-human primate: In many studies, the animals will be prepubertal or peripubertal and evaluation will be difficult at best. In cases where mature females are used, there is a strong social effect on reproduction with subordinate animals displayed a higher incidence of abnormal cycles than dominant animals. This effect can be pronounced when changes in social structure occur (randomisation at the beginning of a study).

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Speaker Abstracts

S10: FEMALE REPRODUCTIVE SYSTEM
Part II: Mechanisms and Patterns of Toxicity in the Female Reproductive System

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Hormonal effects are relatively common, but can be quite variable due to multiple causes and underlying mechanisms. Textbook examples such as the McPhail Assay using immature rabbits and the Type I (atrophic ovary, uterus, and vagina), Type II (atrophic ovary with hyperplastic/hypertrophic uterus and vagina), and Type III (hyperplastic/hypertrophic ovary, uterus and vagina) classification of hormonal effects by Drs. Yuan and Foley are excellent ways to understand the basic effects. However, many cases in the general toxicology setting are not this simple due to weak off-target effects at high doses, direct ovarian effects, stress at high doses, mixed patterns, impact of age, partial agonists, selective receptor modulators, etc.

Administration of compounds that interact with oestrogen, progesterone, or androgen receptors impact the female reproductive system at multiple levels. The direct effects on the uterus and vagina are relatively easy to identify in a general toxicity study. The rodent vagina is especially easy to evaluate with characteristic responses. Oestrugenic compounds produce hyperplasia with keratinisation and progestogenic and androgenic compounds produce distinct mucification (do not confuse with normal prooestrus). In contrast, the ovary can be more difficult to identify changes. This classic Type II response is expected to have an atrophic ovary, but in many cases the ovary does not have a dramatic atrophic appearance. There are typically a number of cycling structures (old corpora lutea and small to medium follicles) and the atrophy is really an absence of recent ovulation and decreased numbers of corpora lutea. This can be hard to detect in the rat especially in short duration studies. In the dog and NHP it is easy to confuse with anoestrus and anovulatory cycles respectively.

Changes in prolactin levels in the rodent are commonly observed and are especially difficult to understand. Dopamine is the main inhibitory factor of prolactin and centrally acting compounds with neuroscience/CNS targets often impact dopamine levels. Prolactin is a major hormone in the regulation of the rodent oestrus cycle and changes in prolactin can have a profound effect on reproduction and the histologic picture observed by the toxicologic pathologist. The difficulty in understanding the effects of prolactin center of the unusual dual role of prolactin in regulating luteal lifespan. In the normal non-mated cycle, the prolactin surge at prooestrus is luteolytic, which leads to the early degenerative changes in the corpora lutea from the previous cycle. If the rat is mated, prolactin production is altered and is actually luteotropic. This explains the fact that both hypo- and hyperprolactinemia lead to an increase in ovarian weight due to the lack of luteolytic peaks and increased luteotropic stimulation respectively.

Alterations in gonadotropins (luteinising hormone and follicle stimulating hormone) can lead to several changes in the ovary including decreased numbers of corpora lutea, follicular cysts, and luteinised follicular cysts. Changes in gonadotropins can be observed secondary to stress.

Decreased production of steroid hormones is another potential mechanism that can have wide reaching effects on the HPG axis and the tubular tract. Inhibition of steroidogenesis can occur at a number of different enzymatic steps (many are specific P450s) and can be either an intended therapeutic target or as a secondary off-target effect. A well know target is inhibition of aromatase, the enzyme that converts androgens into oestrogens in order to decrease endogenous oestrugenic stimulation of oestrogen receptor positive breast cancer. While it might be expected to see a Type I pattern due to decreased oestradiol production, the lack of aromatase activity can lead to an increase in androgenic substrate that would have been converted to oestrogens, which can lead to vaginal mucification and mammary lobuloalveolar hyperplasia, not the expected atrophy. Oestrogens can have both positive and negative feedback effects on gonadotropins and variably luteinised follicular cysts can be observed.

Another interesting hormonal effect is alteration in the timing of reproductive senescence in rodents. The timing of onset of reproductive senescence varies with strain and housing practices with the social stress of isolation during single housing leading to an earlier onset of senescence. This can also occur as a test article related effect. This requires longitudinal assessment to definitively diagnose, but can be observed in 3-6 month general toxicology studies with a very different histologic presentation of the treated animals. In these cases the treated animals look relatively normal for animals going through reproductive senescence, but the timing is off. Note: Exercise some caution with this and do not over-diagnose. A slight difference in the incidence is not likely to be significant due to the high degree of variability.

Direct effects on the ovary (in absence of a primary HPG axis effect) are not uncommon and in the author's experience seem to be more common with compounds being developed for oncology-related indications. The corpus luteum displays impressive in-growth of thecal blood vessels following ovulation and compounds that disrupt angiogenesis lead to central necrosis within the newly forming corpora lutea (do not mistake for newly formed corpora lutea during oestrus/ metoestrus, luteinised follicular cysts, luteal cysts, or luteolysis during normal prooestrus). Pharmacologic inhibition of angiogenesis, as well as other oncology targets, often involves inhibition of a spectrum of receptor tyrosine kinases with differing effects on corpus luteum function and development. In some of these cases, the corpora lutea can be enlarged with an increase in ovarian weight, may be fewer in number, and often contain varying amounts of central haemorrhage, with or without interstitial and/or bursal haemorrhage.

In some cases a spectrum of changes can be observed, which do not fit cleanly into any of the above patterns. In the author's experience this seems to be most commonly observed at or above maximum tolerated doses where there is on-target and off-target effects coupled with systemic toxicity and stress. In these cases, it is typically not critical to understand the mechanism and effort should be placed on evaluating effects on the female reproductive system at doses with minimal systemic toxicity.

The female reproductive system is a challenging area to evaluate due to the inherent variability, but with an organised approach and an understanding of the normal anatomy and physiology a number of basic patterns can easily be observed, which can help in understanding the underlying mechanism. Key points for the toxicologic pathologist include: always evaluate the pituitary, ovary, uterus, cervix, vagina, and mammary gland together and whenever possible use diagnostic terms and try to avoid cycle stages as a diagnosis.

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Speaker Abstracts

S11: PLACENTA OF THE RAT – HISTOLOGY AND PATHOLOGY

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Healthy placental growth and function are essential for normal embryonal and fetal development and growth. Xenobiotics directly or indirectly altering the morphology and functionality of the placenta may induce embryonal/fetal lethality.

In several studies conducted at BASF SE with Epoxiconazole, the placentas at term in rats were increased in size and had significantly increased mean weights and late resorptions.

Little is known about histopathological alterations in the placenta of laboratory rodents. Therefore placentas from rat dams were examined by light microscopy in modified prenatal toxicity studies with Epoxiconazole. Histopathology of the placentas revealed dose-dependent degenerative changes in the labyrinth and trophospongium. The placenta histopathology offered valuable insights on the underlying mechanism for the observed postimplantation losses and triggered further investigations to clarify the human relevance of the rat findings.

The results generated through different study designs are presented here and put into context with the time course of normal placental development in rats.

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Speaker Abstracts

S12: THE NON-HUMAN PRIMATE PLACENTA

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The placenta fulfills basically identical functions in all placental species – embryo-/fetal nourishment, homeostasis, and protection. It is otherwise considered as one of the most variably structured organs among species including rodents and NHP which represent the most relevant species for humans in toxicological research (Kaufmann, 1990; De Rijk and van Esch, 2008; Benirschke, 2012). The placenta is a pivot on medication of pregnant woman which exerts wide impact on the unborn child. It may inhibit, allow, or modify the applied compound on reaching the fetus. However, the underlying mechanisms of a triggering placenta function often remain in the dark and the effect of an applied compound uses to be concluded from normal or abnormal development of the fetus/newborne animal. The placenta is a temporary changing organ, not present from the beginning of pregnancy, and consequently its function as a compound mediator alters during gestational time. Such certainly is of high concern in toxicological research and in the estimation of compound effects. In the presentation we discuss morphological and functional aspects of the primate placenta with special emphasis on the cynomolgus monkey as a model in human toxicological research.

The structure of the cynomolgus monkey placenta appears immature in late embryogenesis of gestation day 50 (GD 50). Both cellularity and vascularisation are incompletely structured and the mature feto-maternal blood borne metabolism has not yet gained a final status. Immaturity furthermore involves numerous physiological parameters, as are the trans-placenta transport mechanisms and the transport of IgGs (Buse et al., 2014). The question of placenta trans-membrane transport has rather gained toxicological urgency since applying IgGs as tools for diagnostic purposes or as immunotoxico-therapeuticals (e.g. mabs: infliximab, omalizumab; Pentsuk et al. 2009). IgG trans-membrane transport is a FcRn-mediated active, specific, and affinity restricted process in many/most placental animals. In the cynomolgus monkey the IgG trans-membrane transport obviously resembles transport in humans, whereas there are fundamental differences in rodents (Landor, 1995). The placenta structure shows relative advanced maturity on GD 100, including the villus cell load and vascularisation as well as the expression of the structural proteins desmin, vimentin, actin, and P11 (S100 A10).

Degenerative criteria, such as trophoblast lesions and fibrinoids become increasingly obvious until birth (GD 160).

On the endocrine context the cynomolgus monkey placenta maturation is exemplified by the ovary and placenta borne hormone progesterone, which is produced in relative high quantity of up to 100 nmol/L. Progesterone mediates a complex body of all maternal, placental, and fetal functions, partly not fully understood yet. Whereas its classical role relaying a relaxing effect on the uterus muscles to prevent labor, it is now obvious that there are further direct and indirect tasks. Upon interference with Annexin II and P11 (S100 A10) progesterone mediates trans-placenta transport mechanisms (via FcRn) as well as immunological functions of materno-fetal acceptance.

Mediation of the feto-maternal immune conflict has to be considered as one of the protective key roles of the placenta. Toxicologically it is of special relevance in view of immune stimulating and suppressive applications. The allograft character of the fetus urges tolerance from the maternal immune system. After former theories of e.g. an immune privileged uterus compartment or reduced immunogenicity of the fetus, we nowadays favour the role of certain fetus borne immune depressing/modifying molecules (Thellin et al. 2000). In the cynomolgus monkey the underlying mechanisms appear largely similar to the human situation but they are by no means completely identical. HLA-DR, HLA-G, Fas-ligand (CD95), and IDO have been identified by application of antibodies which are also used in humans. On GD 50, the Fas-ligand and Annexin II are expressed in their final positions in the cynomolgus monkey, whereas they are described in different topography in humans. On GD 100, most feto-maternal tolerance parameters tested so far are in place (progesterone, HLA-DR, HLA-G, IL5, IDO). Moreover, at that age the placenta villi become increasingly occupied by fetal immune derived cells, due to the generation of the genuine fetal immune system (Buse, 2005; Buse et al. 2014).

The findings made so far in the cynomolgus monkey demonstrate a high but not a complete level of similarity with human placenta (species specificity). Placenta maturation comprises the stepwise development of a barrier function (age dependency of trans-placenta control). Consequently, an interventional capability is considered to alter time dependently.

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Speaker Abstracts

S13: STABILISED NON-IMMUNOGENIC MESSENGER RNA FOR TRANSCRIPT THERAPY

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Replication-deficient viruses have been used successfully in the field of gene therapy because of their high transfection efficiency. However, the risk of insertional mutagenesis and induction of undesired immune responses remain critical obstacles for their safe medical application. On the other hand, nonviral vectors have been intensively investigated for plasmid DNA (pDNA) delivery as a safer alternative, although their gene transfer efficiency is still many folds lower than for viral vectors, which has been predominately attributed to the insufficient transport of pDNA into the nucleus. Instead of pDNA, messenger RNA (mRNA) has recently emerged as an attractive and promising alternative in the field of nonviral gene delivery. This strategy includes several advantages compared to the use of pDNA: i) the nuclear membrane, which is a major obstacle for pDNA, can be avoided because mRNA exerts its function in the cytoplasm; ii) the risk of insertional mutagenesis can be excluded; iii) the determination and use of an efficient promoter is omitted; iv) repeated application is possible; v) mRNA is also effective in non-dividing cells, and vi) vector-induced immunogenicity may be avoidable. In particular, immunogenicity concerns have been successfully solved by inclusion of chemically modified nucleotides in the mRNA molecule during in vitro transcription. In our approach, replacement of 25% of uridine and cytidine by 2-thiouridin and 5-methylcytidin, respectively, largely reduced mRNA binding to sensors of the innate immune system, including Toll-like receptors and RIG-I, which largely reduced mRNA immunogenicity in vivo after intravenous and intrapulmonary application. According to these characteristics, we termed these chemically modified mRNA stabilised non-immunogenic messenger (SNIM®) RNA which are now commercialised by the company ethris GmbH, for development of "Transcript therapies" for the treatment of various inherited diseases and application in regenerative medicine.

The potential range of programs to which the technology can be applied is very broad spanning metabolic or hereditary monogenetic disorders to regenerative medicine. We have successfully developed technologies for the pulmonary delivery of SNIM® RNA, thus demonstrating SNIM® RNA products can be delivered conveniently and efficiently by a number of routes that will support patient compliance and quality of life.

Poster Abstracts

ESTP Poster Abstracts

TP01: Implementation of the INHAND nomenclature: the Janssen experience

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The sustained publication of INHAND rodent organ or organ system histopathology glossaries in Toxicologic Pathology and Journal of Toxicologic Pathology (with the last versions available on www.goreni.org), combined with the soon-to-come preferred use of this nomenclature in dossiers submitted to FDA under SEND format, prompted us to harmonize our Janssen internal glossary with the new nomenclature.

Based on the INHAND documents already published (one issue on basic principles and 8 issues on organ / organ systems, prepared by different Working Groups) and on the Basic Principles dated 21 November 2005 and available on the ESTP and NA-STP websites, we listed the INHAND glossary entries which did not follow the basic principles, and also the differences with entries present in Janssen glossary or in existing sources.

Here are some observations following our analysis:

- The use of descriptive term is preferred to the process term. However, some Working Groups use “Thrombosis”
- In examples like “Alveolar emphysema” or “Glandular metaplasia”, modifiers should appear after a base term
- Depending on the organ, “Ectasia” or “Dilation” is present in the base terms while the basic principles require that similar lesions should always have the same base term across organs
- Compound-term should not mix unrelated processes like in “Pigments, dusts, inert materials”
- Some disease terms or etiologic agents are used as base terms, like “Tyzzer’s disease” or “Ectoparasites”.

The poster will detail more examples and will also include our proposals.

This kind of careful assessment of the glossaries, as standalone documents and by comparison with existing sources, is certainly useful to complete and improve INHAND nomenclature. An INHAND Change Control procedure curated by the Global Editorial Steering Committee (GESC) has been put in place, and its role is to update regularly the nomenclature. Every user can propose updates which, when approved, will appear in goRENI and will be posted on society websites. We intend to present our comprehensive analysis to the GESC, and a companion paper is under preparation in order to share our experience with the community.

We encourage all pharmaceutical companies to perform this work of comparing their own glossary to the INHAND nomenclature. This will allow pathologists to gradually adapt to this new glossary, and to prepare for the SEND formatting of reports that FDA will require probably during 2015.

ESTP Poster Abstracts

TP02: The National Toxicology Program Nonneoplastic Lesion Atlas

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The National Toxicology Program (NTP)'s Nonneoplastic Lesion Atlas is a valuable, web-based resource with thousands of high-quality, enlargeable images, diagnostic guidelines, and preferred NTP terminology for numerous nonneoplastic rodent lesions. The atlas will be used by the NTP and its many pathology partners to standardise lesion diagnosis, terminology, and the way lesions are recorded in NTP studies. The goal is to improve the consistency and accuracy of the diagnosis of nonneoplastic lesions between pathologists and laboratories to improve the organisation and utility of the NTP's nonneoplastic lesion database and, ultimately, our understanding of nonneoplastic lesions.

The NTP Nonneoplastic Lesion Atlas is a living document that complements the INHAND publications. In fact, one of the aims of the atlas is to align the NTP terminology with that of the INHAND publications as much as possible. The atlas is also a useful training tool for pathology residents and can be used by any organisation to improve their own nonneoplastic lesion database. A total of 56 organs organised into 13 organ systems will be included in the completed project.

The atlas is free to the public at http://ntp.niehs.nih.gov/nnl.

ESTP Poster Abstracts

TP03: A SEND solution for microscopic pathology, in-life, and other preclinical toxicology: data collected using multiple LIMS (Laboratory Information Management Systems)

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Toxicology studies in today's business environment involve multiple testing sites and LIMS. For example, it is not uncommon for microscopic pathology data to be collected on a different LIMS or at a different site from that used for in-life. SEND solutions therefore need to harmonize potential heterogeneity of metadata, file formats, and terminology to produce one SEND dataset that is consistent across domains. Moreover, all FDA-compliant SEND datasets need to include appropriate correlations, such as macroscopic and microscopic findings and, if appropriate, palpable masses and clinical observations, regardless of LIMS or study sites used. For carcinogenicity studies, microscopic and tumor data need to be mapped to SEND tumor.xpt files.

We wish to report a web-based software architecture for SEND that accomplishes the aggregation, harmonization, and translation of data from different sources into one complete SEND dataset including all XPT files, define XML and PDF files, and validation reports. The architecture involves input from LIMS-specific or file-based adaptors, which are then processed through an engine that maps data to appropriate SEND variables and domains; harmonizes metadata between different domains, such as animal and study number; consolidates comments (co) and establishes relationships (relrec); and performs controlled terminology mapping. Trials domains, which are the *planned* features of a study in standardised format and required for every SEND dataset, can be easily tailored to sponsors' business needs in a SEND-compliant fashion through the use of Trials Expert Tools. The architecture supports easy versioning as new releases of SEND and CDISC controlled terminology (CT) become available. CT mappings from previous CT releases that need to be maintained for new CT releases are preserved, so that only changes in CT from release to release need to be mapped.

This SEND solution has been used successfully to submit SEND datasets created by multiple source systems to the FDA, including different LIMS for in-life and microscopic pathology. We conclude that it is possible and necessary to develop SEND solutions for preclinical studies involving multiple LIMS.

ESTP Poster Abstracts

TP04: The RITA Database – incidences of preneoplastic and neoplastic lesions in young animals

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Introduction: Tumours in young animals are considered as a relatively rare event. However, their presence might evoke concerns especially when they occur in treated animals in short-term studies. There are a few publications available that address this topic (see references).

Methods: The RITA database contains historical control data of peer-reviewed histopathological diagnoses of tumours and other proliferative lesions. The database reflects data from more than 26,000 rodents of carcinogenicity studies. Animals which died or were euthanized moribundly at an age younger than 6, and between 6 and 12 months were selected from the database and compared to animals older than 12 months. Incidences of preneoplastic and neoplastic lesions of these three groups were compared to each other. Preneoplastic lesions were defined to be focal or diffuse hyperplasias which represent differential diagnosis to tumours.

Results: The incidence of preneoplastic lesions of rats younger than 6 months was 9%, at an age between 6 and 12 months 25%, and in rats older than 12 months 78%. For mice the incidences were 5% in animals younger than 6 months, 11% for animals between 6 and 12 months of age, and 39% for animals older than 12 months. The earliest tumor occurring was an adenoma of the distal part of the pituitary gland in a 61 days old female Wistar rat. The tumor incidence of rats younger than 6 months was 18%, at an age between 6 and 12 months 44%, and in rats older than 12 months 80%. For mice the incidences were 12% in animals younger than 6 months, 23% for animals between 6 and 12 months of age, and 60% for animals older than 12 months.

Discussion: Interestingly, the incidences of tumours were always high compared to incidence of peneoplastic lesions. Since tumours do occasionally occur already in young animals historical control data is also needed for these incidences. Therefore, the RITA group is planning to include in the future tumor and pre-neoplastic incidences from studies with shorter duration (e.g. 13 or 26 weeks). Further knowledge about incidences of preneoplastic and neoplastic lesions of young animals will improve evaluation of short-term studies.

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ESTP Poster Abstracts

TP05: Nephroblastomas and nephroblastematosis in young Sprague-Dawley rats

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This report describes the occurrence of two nephroblastomas in each of two recent rodent toxicity studies in Crl:CD (SD) rats (1 female and 3 males, one low dose animal and 3 vehicle control animals, all 4 on the right kidney). In addition we describe the occurrence of a related condition we preferred to term nephroblastematosis in a young rat of the same strain and the features which are suggested to differentiate this lesion from nephroblastoma.

Grossly, in all 4 animals the nephroblastomas were unilateral, solitary masses ranging from 3-5 cm in diameter. The morphological features of the nephroblastomas were those classically described with varying proportions of sheets of blastemal cells and the formation of primitive glomerular like structures and tubules.

In contrast to the above nephroblastomas, the one case diagnosed as nephroblastematosis was bilateral, multifocal and without a single defined principal mass. The foci were distributed throughout the cortex, principally in the mid-cortical region. Most foci consisted solely of blastemal cells which infiltrated between normal appearing tubules, but occasional glomerular-like structures were present in some larger foci. The term nephroblast*o*matosis is another variant described in the human literature and our case bears many similarities to this entity.

According to the literature, nephroblastomas are uncommon tumors in Sprague-Dawley rats. One large retrospective review did not report any nephroblastomas in control animals from 20 consecutive carcinogenicity studies. In data published by Charles River, nephroblastoma was diagnosed in 1 of 2146 control male Crl:CD (SD) rats and none in control females in 30 studies. None of these publications make mention of nephroblastematosis.

The significance of our observations therefore lies in the fact that compared to the literature on this strain of rat and previous in- house historical data; this represents a significant increase in the incidence of this tumor, albeit over a short period of time. Because this tumor can also be experimentally induced, the historical incidence can be an important aid in differentiating between a treatment related effect and spontaneous occurrence.

With regard to nomenclature, these conditions seem to all be part of the same process and the distinction, in many cases, would seem arbitrary.

ESTP Poster Abstracts

TP06: Immunohistochemical characterisation of ENU-induced brain tumors in F344 Rats

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Introduction: N-ethyl-N-nitrosourea (ENU) is an alkylating agent that is used extensively in experimental neuro-oncogenesis. In rats, ENU causes neural neoplasms in up to 100% of offspring of dams given a single dose in the second half of gestation. Experimental Design: Immunohistochemical staining was performed to further characterise 26 ENU-induced brain tumors from 19 animals selected from a study in which 20 pregnant Fischer 344 rats were given 20 mg/kg ENU intravenously 4 days before giving birth. The original diagnoses were based upon evaluation of hematoxylin & eosin stained sections and consisted of 8 glioblastomas, 11 astrocytomas, and 7 oligodendrogliomas.

Materials and Methods: Hematoxylin & eosin slides were prepared from each animal. Immunohistochemical stains included glial fibrillary acidic protein (GFAP), ionized calcium-binding adapter molecule 1 (Iba-1), and oligodendrocyte transcription factor 2 (Olig2).

Results: A total of 19 tumors were observed and diagnosed as oligodendrogliomas based upon immunohistochemical evaluation. Of the 7 animals with more than 1 brain tumor originally diagnosed, these were considered the same tumor based upon immunohistochemical evaluation and were given 1 diagnosis. Neoplastic cells stained most consistently with Olig2. here were 5 well-differentiated oligodendrogliomas. The remaining 14 tumors exhibited considerable cellular anaplasia and corresponding variability in Olig2 staining. These tumors also contained greater numbers of Iba-1+ microglial cells (diffuse) and GFAP+ astrocytic cells (largely distributed at the tumor edge and around blood vessels). The "ependymoma-like," pseudorosette features of ENU-induced tumors stained positively for Iba-1, suggesting reactive microglia/macrophages.

Conclusion: ENU-induced neoplasms consisted of variably differentiated oligodendrogliomas based upon immunohistochemical evaluation. While some tumors were well-differentiated, others were anaplastic with robust secondary infiltrates of reactive cells (microglial, astrocytic, endothelial).

Impact Statement: A previous study indicated most spontaneous tumors in the rat were oligodendrogliomas, while chemically-induced neoplasms were malignant microglial tumors. Results for ENU are unique thus far for its induction of variably differentiated oligodendrogliomas.

ESTP Poster Abstracts

TP07: Proliferative lesions of the endocrine system in 600 control Han: AURA Syrian hamsters from 24-month toxicity/carcinogenicity studies

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Introduction: Syrian golden hamsters (*Mesocricetus auratus*) are much less often used in toxicity and carcinogenicity studies than rats and mice. However, hamsters may serve as a valuable alternative animal model for certain compounds and experimental designs. A thorough knowledge on the background pathology of this species, e.g. of the endocrine system, is very helpful for a correct interpretation of study findings, although the concurrent control group in toxicity/carcinogenicity studies still remains the most important comparator for identification of possible treatment-related effects.

Material and Methods: The presented incidences of tumors and pre-neoplastic proliferative lesions were pooled from 600 Han: AURA hamsters from the Fraunhofer ITEM breeding colony which served as untreated cage controls (240 of each sex) or as vehicle controls (60 of each sex) in long-term toxicity/carcinogenicity studies conducted between 1988 and 2009. While 100 control animals were from a chronic inhalation toxicity study, 500 hamsters served as controls in oral toxicity/carcinogenicity studies. From these 500 hamsters, incidences on spontaneous neoplasms and common non-neoplastic lesions have recently been published: *McInnes, Ernst and Germann: Tox Path 41: 86-91, 2013; Tox Path, doi: 10.117/0192623314532569, 2014*.

All hamsters were aged about 6 weeks at start of studies and kept under standard laboratory conditions: housed individually in type-III Makrolon (polycarbonate) or metal wire mesh cages (inhalation study), maintained on a 12 h light/dark cycle, at 22 ± 2 °C, at 40-70% relative humidity and with free *ad libitum* access to tap water and a pelleted diet. All animals were necropsied and a histological examination of all organs was conducted.

Results: In all 4 hamster studies, males outlived females. The average mortality rate at 24 month was 33% in males and 65% in females. With 78% in males and 79% in females, the percentage of tumor-bearing animals was nearly similar in both sexes. The majority of tumors and pre-neoplastic lesions in all 4 studies originated in the endocrine system. In the adrenals, 281/161 cortical neoplasms, 3/3 medullary tumors, 310/231 cortical hyperplasias and 4/5 medullary hyperplasias were observed in males/females. Other endocrine organs were much less affected. In the pituitary, 5/26 pars distalis tumors, 4/16 pars distalis hyperplasias and 1/0 pars intermedia hyperplasia occurred in males/females. In the parathyroids, 5/30 adenomas and 9/43 hyperplasias were diagnosed in males/females. The thyroids revealed 4/7 follicular adenomas, 5/7 C-cell tumors, 5/19 follicular cell hyperplasias and 5/13 C-cell hyperplasias in both sexes. In addition, 1/2 ectopic C-cell tumors and 2/3 ectopic C-cell hyperplasias were detected at the laryngo-tracheal junction. In the pancreas, 15/12 islet cell tumors and 28/13 islet cell hyperplasias were observed in males and females, respectively.

Conclusion: Adrenal cortical adenomas and carcinomas are the most commonly observed tumor types in Han: AURA hamsters. They may be observed already at an age of 6 months. Adrenal cortical tumors also arise at a much higher frequency in hamsters than in rats and mice. As in mice, a differentiation between cortical adenomas and subcapsular cell adenomas of the adrenal cortex can also be done in hamsters due to morphological differences in the cell types involved. The tumor prevalence in the other endocrine organs is not higher compared to rats or mice. Due to the high mortality in females at 24 months, it is recommended to confine long-term toxicity/carcinogenicity studies in hamsters to 18-20 months duration.

ESTP Poster Abstracts

TP08: Spontaneous diffuse large B-cell lymphoma in a cynomolgus monkey (*Macaca fascicularis*)

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INTRODUCTION: There are only few case reports of spontaneous non-viral associated lymphomas in cynomolgus monkeys. In the present case we describe a diffuse large B-cell lymphoma in a naïve, male, six year old *Macaca fascicularis*.

CASE DESCRIPTION: A naïve, male, six year old cynomolgus monkey (*Macaca fascicularis*) developed a rapidly progressing subcutaneous mass on the lateral right shoulder extending into the proximal part of the upper arm. Due to the invasive nature of the mass, a performed surgical excision remained incomplete. Samples of the mass were sent for histopathological evaluation.

Histopathology revealed an infiltrative, growing mass of blastoid medium sized cells with large nuclei and prominent nucleoli, a small rim of eosinophilic cytoplasm, moderate anisokaryosis and up to 8 mitotic figures per high power field. The center of the mass was necrotic. In immunohistiochemistry, the cells stained positive for CD20 (B-cell origin). In addition, the mass was infiltrated by macrophages (CD68 positive) and T-lymphocytes (CD3 positive). In addition, a fine needle aspiration was taken from the enlarged right deep cervical lymph node showing up to 70% medium sized to large round lympho-blastoid cells. These cells had prominent nucleoli and displayed moderate karyomegaly, anisokaryosis and anisocytosis with occasional mitotic figures. In hematology, the animal showed a minimal and insignificant increase in neutrophilic granulocytes only.

Due to the poor prognosis the animal was euthanized and sent to necropsy 23 days later. At necropsy, the original surgical site had completely healed and no residual tumor tissue could be detected histopathologically. However, the right deep cervical lymph node and the right axillary lymph node were massively enlarged with necrotic areas in the center. Histologically, these lymph nodes showed large areas of central necroses surrounded by a rim of blastoid cells, macrophages and small lymphocytes. In addition, remnants of these cells were present within the necrotic mass. Similarly to the biopsy, the cells stained positive for CD20 (B-cell origin) in immunohistiochemistry intermixed with some macrophages (CD68 positive) and T-lymphocytes (CD3 positive). These findings are consistent with a diffuse large B-cell lymphoma.

Other lymphatic tissues, bone marrow and remaining tissues showed no histopathological finding or only minimal findings consistent with normal background lesions regularly seen in cynomolgus monkeys.

The animal has been tested negative repeatedly for the presence of infectious agents including simian retrovirus (SRV), simian immunodeficiency virus (SIV) and simian T-lymhotropic virus (STLV) in the context of the internal health monitoring program for non-human primates.

CONCLUSION: Based on the histological picture and supported by immunohistochemical staining of the respective cells a diffuse large B-cell lymphoma was diagnosed in a naïve, male, six year old *Macaca fascicularis*.

ESTP Poster Abstracts

TP09: Induction of malignant mesotheliomas by intraperitoneal injection of carbon nanotubes in rats

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Introduction: Biological effects of tailor-made multi-walled carbon nanotubes (MWCNTs) were investigated *in vivo* in a 2-year carcinogenicity study in a project funded by the German Federal Ministry of Education and Research (contract no. 03X0109A).

Methods: Fifty male Wistar rats per group (total of 500 rats) were treated once by an intraperitoneal (i.p.) injection of a low ($1x10^9$ WHO fibers) and high ($5x10^9$ WHO fibers) dose each of MWCNTs (MWCNT 1, 2, 3 and 3a) suspended in artificial lung medium, which was also used as negative control. Amosite asbestos ($0.1x10^9$ WHO fibers) served as positive control. Moribund rats were sacrificed and necropsy comprising all organs was performed.

A histopathological classification of tumors and in addition, immunohistochemistry was conducted for podoplanin, Wilm's tumor antigen 1, cytokeratin, vimentin, desmin, smooth muscle actin, ICAM-1, CD146, thrombomodulin, matrix metalloproteinase 14, CD90, CD147 and Ki-67 to compare the induced tumors with mesotheliomas occurring in humans.

Results: The treatments induced mesotheliomas in all dose groups, whereas incidence and time to tumor were different between the groups. Rats treated with MWCNTs 3 (L = 8.57 µm; D = 0.085 µm) or 3a (L = 9.3 µm; D = 0.062 µm) were killed moribund between 8 and 12 months after treatment and exhibited high tumor incidences in the low and high dose groups. The survival time of rats treated with MWCNT 2 (L = 10.24 µm; D = 0.04 µm) was longer but resulted also in high tumor incidences in both dose groups in relation to the positive control. For MWCNT 1 (L = 7.9 µm; D = 0.037 µm) the survival time for the low dose was similar to the amosite asbestos (L = 13.95 µm; D = 0.39 µm) positive control. Overall, tumor incidences were lower than for MWCNTs 2, 3 and 3a. Most tumors were histologically and immunohistochemically classified as malignant mesotheliomas.

Conclusion: Tumors induced by i.p. injection of different MWCNTs and of asbestos were histopathologically and immunohistochemically similar, also in comparison to mesotheliomas in men, suggesting a similar pathogenesis. Based on our results of the carcinogenicity study with MWCNTs the accreditation and production of CNT-fibers has to be regulated urgently.

ESTP Poster Abstracts

TP10: Comparison of pulmonary inflammation after short-term (5 days and 4 weeks) inhalation exposure to CeO_2 nanoparticles based on analysis of bronchoalveolar lavage fluid and histopathology

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The use of nanoparticles in biomedical and industrial applications requires the identification of human hazard potential of air-born nanoparticles. Poorly soluble nanoparticles (PSP) have shown to elicit pulmonary inflammation in rats after inhalation. As representative of PSP, CeO_2 is tested in short-term inhalation studies (5 days and 4 weeks of exposure) which provide information on biokinetics and biological effects required for the design of a currently investigated chronic and carcinogenicity study. The CeO_2 is coded as NM-212 in the list of OECD WPMN Sponsorship Programme for the Testing of Manufactered Nanomaterials. Part of the work is funded by the EU-projects NanoREG and NanoMILE.

Female Wistar (Crl:WI(Han)) rats were whole-body exposed to 0.5, 5 and 25 mg/m³ CeO_2 for 6 h/d for 5 days and 4 weeks. Lung and lymph node burdens, broncho-alveolar lavage (BAL) and complete histopathology were assessed after exposure and after an exposure free period up to 129 days.

Inflammatory potential was compared after 5 days and 4 weeks of exposure using the two different methods (BALF analysis and histopathology). After both studies, an increase of biochemical and cytological parameters (e.g. neutrophils, lymphocytes) were observed in BAL fluid in a concentration-related manner. This indicates a moderate inflammation in the lung which regressed over post-exposure period. Histopathology revealed macrophage-particle interactions resulting in substance- and concentration-related morphological changes (alveolar histiocytosis, macrophage aggregates) in the lungs and lung associated lymph nodes in both studies. Granulomatous inflammations ensued to longer (4 weeks) exposure and post exposure time. Furthermore, incidences and grading of findings were affected by duration and amount of particle burdens in the lung. Regression of BALF parameter and progression of histopathological findings over time are presented here.

ESTP Poster Abstracts

TP11: The value of cell proliferation measurement for early detection of carcinogenic potential of carbon nanotubes after intraperitoneal injection in rats

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Introduction: Multiwall carbon nanotubes (MWCNT) are discussed to have a toxic potency depending on their length and fiber-like shape. To investigate potential early recognition of carcinogenic behavior of MWCNT, they were injected intraperitoneally in rats and the cell proliferation rate was measured at the diaphragmatic peritoneum in a project funded by the German BMBF.

Methods: Tailor-made MWCNT (1, 2, 3) with different lengths and diameters were produced, suspended in artificial lung medium and injected intraperitoneally in rats in two dose groups (low: $1x10^9$ WHO-fibers; high: $5x10^9$ WHO-fibers). Long amosite asbestos ($0.1x10^9$ WHO-fibers) served as positive controls, ground MWCNT (3) and Printex 90 (5 mg/rat) as particle negative controls. Three, 6 and 12 months after injection of fibers, the animals were necropsied, the thickness of the peritoneum at the diaphragm was measured, and the cell proliferation rate was determined using the BrdU method. Histopathological examination of the diaphragm was performed to detect possible tumors. Furthermore, the acquired data were correlated with a parallel conducted carcinogenicity study.

Results: There was a time-independent significant increase in the cell proliferation rate of MWCNT $1(_{high})$ (length=7.9µm; diameter=0.037µm), MWCNT $2(_{low/high})$ (length=10.24µm; diameter=0.04µm) and MWCNT $3(_{low/high})$ (length=8.57µm; diameter=0.085µm) comparable to amosite asbestos (length=13.95µm; diameter=0.39µm). The peritoneal thickness of the diaphragm increased time-independently in animals treated with MWCNT $2(_{low/high})$ and MWCNT $3(_{low/high})$. Injection of MWCNT 1 caused after 12 months a significant increase only in the high dose group.

Histopathologically, after 12 months all animals treated with MWCNT $3(_{low/high})$ or CNT $2(_{high})$ (n=5) showed mesotheliomas. In addition, in three animals treated with CNT $2(_{low})$ mesotheliomas were induced. Similarly, MWCNT 2 and 3 showed a higher mesothelioma incidence than MWCNT 1 in the carcinogenicity study conducted in parallel.

Conclusion: Some MWCNT mediate enhanced proliferation of peritoneal cells of the diaphragm in rats which correlated well with results of the parallel carcinogenicity study. Early detection of carcinogenic potential of CNT via cell proliferation measurement of the diaphragmatic peritoneum after intraperitoneal injection in rats is possible and represents a valuable method, which should be implemented in the systematic evaluation of nanofiber toxicity.

ESTP Poster Abstracts

TP12: Toxicity studies with nanoparticles and detection methods for pathology evaluation

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Nanoparticles including nanotubes are currently under special considerations for their toxicologically relevant reactions. Hereby, a major issue is traceability in cells and tissues that is triggered by their size, elasticity and mechanical behavior. Especially the latter is of a big concern, e.g. nanotubes may be extremely flexible causing coils that may settle in cells, or nanoparticles with the potential of bio adhesion that causes entering into mucus overlaying cell layers, getting endocytosed etc.

To understand new ways to trace nanoparticles within tissues, several methods have been set up by the authors.

For in-vitro studies, an in-life videography method by a laser technology was established to observe the ability of macrophages to ingest nanotubes.

In addition, a validation inhalation toxicity study was performed. Test item was evaluated as free test item, on inhalation filters, as well as in tissues by different methods.

Changes in functional and enzymatic parameters were observed in broncho-alveolar lavage fluid.

Hyperspectral nanoscale analysis was used to trace even minimal quantities within macrophages and in connective tissue of animals from inhalation studies. The results were compared by evaluation of routine sections. This was supported by REM and laser scanning microscopy on unstained paraffin sections, Epon embedded material, and by a new method called ‘dried thin tissue sections. Furthermore, EDX analysis was performed on all available material. In addition, low temperature ashing was applied to regain nanomaterials in ashes to establish possible methods for quantifications.

ESTP Poster Abstracts

TP13: Two unusual background lesions in the central nervous system of rats

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Introduction: Differentiation of background lesions from treatment related changes is one of the major challenges for a pathologist working in the field of toxicology. This poster presents two unusual central nervous system (CNS) findings in rats.

Case presentation: A 28-day oral repeated dose toxicity study was performed in Wistar Han rats. In the histopathological examination two animals had unusual findings in the cerebellum and pons.

Case 1: A female rat in the high dose group had several foci of vacuolation and gliosis in the white matter of the cerebellum, near the area of deep cerebellar nuclei. The overlying cerebellar cortex showed multifocal degeneration and necrosis of Purkinje cells and granular cell layer. The cause for the lesion could not be determined based on the tissue sections, but was most likely related to an ischemic insult (i.e. local disturbance of the circulation).

Case 2: A female rat in the middle dose group had a focal well-demarcated cellular aggregate in the pons, beneath the fourth ventricle and the cerebellum. The lesion consisted of a central densely cellular core, surrounded by a corona-like perpendicular cell layer. The cellular origin of the lesion was not confirmed, but most likely it is a developmental disorder of the cerebellum (i.e. ectopic cerebellar cortex).

Discussion: Background lesions in the CNS of rats are uncommon in short term toxicity studies and usually mild in severity. In these two cases the lesions were severe and extensive. However, no other CNS lesions were seen in the rest of the study animals and the test compound had no known effect on the CNS. Based on the low number of affected animals, the different dose levels of the affected animals, and the different histological type of the lesions, the findings were considered to represent background lesions not related to the treatment with the test compound.

ESTP Poster Abstracts

TP14: Fatal fasting syndrome in a cynomolgus monkey (*M. fascicularis*)

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A 69-month old female cynomolgus monkey (*Macaca fascicularis*) was found in poor clinical condition and was euthanized one week after reception from the supplier (before being placed on a study). At clinical examination on the day of necropsy, the monkey was found hypothermic (32.4°C), markedly dehydrated, pale and prostrate.

Hematological and biochemical abnormalities included prolonged clotting times (high APTT and PT), low sodium, chloride and calcium and high phosphorous concentrations, hypoglycemia, high BUN and creatinine, hypertriglyceridemia, hypoalbuminemia (causing hypoproteinemia and low A/G ratio), high ASAT and lipase and low γGT.

At necropsy, the abdominal fat was prominent, with multiple white/grey foci and adhesions to the kidneys, pancreas, liver, spleen, adrenals, stomach, intestine and abdominal wall. A few grey foci without adhesions were also observed in the diaphragm. The kidneys were pale and the liver was enlarged and pale.

Histopathologically, major changes were observed in the pancreas, liver, kidneys and abdominal fat. In the pancreas, there were multiple foci of acute acinar necrosis associated with acinar and periacinar edema, fibrin deposits, haemorrhages and acute inflammatory cell infiltration. The acinar pancreatic units were distorted and the acinar cells had enlarged nuclei and weakly stained cytoplasm with decreased zymogen granules. Cell debris was seen in the excretory ducts. There were multiple large foci of necrosis and granulomatous inflammation in the peripancreatic fat. In the liver, there was a severe panlobular hepatocellular macro-vacuolation. In the kidneys, there was an extensive macro-vacuolation of proximal convoluted tubular cytoplasm and scattered foci of interstitial fibrosis, tubular basophilia and mineralisation There was no evidence of necrosis in the liver or kidney. The vacuoles were Oil-Red-O positive. Other histopathological changes included necrosis, fibrin deposits and acute or granulomatous inflammation in the adipose tissue surrounding the heart and several abdominal organs, and in the submucosal fat of the cecum. Granulomatous fat inflammation was also observed near the biceps femoris muscle, which additionally showed chronic inflammation with fiber necrosis, interstitial haemorrhages and fibrosis. Other changes were seen in the esophageal mucosa (ulceration and acute inflammation) and mandibular salivary gland (decreased secretory granules).

Overall, these pathological changes, especially the severe liver and kidney lipidosis, the pancreatic inflammation and the fat tissue changes were consistent with Fatal Fasting Syndrome, which has been described as occurring occasionally in cynomolgus monkeys as well as other non-human primate species [1,2].

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ESTP Poster Abstracts

TP15: *Trypanosoma cruzi* infection (Chagas disease) in cynomolgus monkeys (*Macaca fascicularis*) obtained from a primate facility in Texas, U.S.A.

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Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi*, is a potentially life-threatening disease that affects an estimated 7 to 8 million people. Approximately 30 to 40% of infected people develop associated cardiomyopathy and/or digestive megasyndromes. The disease is limited to the Western Hemisphere and is most prevalent in Latin America, although in recent decades it has been increasingly detected in the southern United States. *Trypanosoma cruzi* is primarily transmitted to humans and animals via triatomine bug vectors.

Over the span of 28 months during 2011-2013, *Trypanosoma cruzi* infection was diagnosed during routine histopathologic evaluation in six cynomolgus monkeys (*Macaca fascicularis)* from separate nonclinical safety studies at our facility in Michigan. Since the organism and vector are not endemic to either Michigan or the countries of origin of the monkeys, infection was presumed to have occurred during the holding period at a Texas primate facility. The primary histologic lesions in these animals included lymphocytic myocarditis and myositis of the gastrointestinal muscularis with involvement of the myenteric plexus. Rare intracellular protozoan parasites consistent with amastigotes of *T. cruzi* were identified in muscle cells in all monkeys. Formalin-fixed paraffin-embedded sections of heart from two affected monkeys were submitted for polymerase chain reaction (PCR) testing and were positive for *T. cruzi*.

Since *T. cruzi* infections have the potential to mimic/obscure various toxicities, amastigotes are not always associated with the lesions, and false negatives can occur with PCR testing of formalin-fixed tissue, we now require screening at the holding facility, with delivery of only PCR and serology-negative animals. Subsequently, no additional cases have been observed at our facility.

ESTP Poster Abstracts

TP16: *Cysticercus fasciolaris* infection in Korean wild rats (*Rattus norvegicus*) and formation of cysts by timely remodeling of collagene types

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Cysticercus fasciolaris, the larva of *Taenia taeniaeformis,* is commonly encountered in rodents. In this study, 287 wild rats *(Rattus norvegicus)* in South Korea were examined in 2010 and 2011. Of 287 rats, 97 (33.8%) were infected with *C. fasciolaris*. A strong positive correlation was found between the host body weight and prevalence in both sexes, regardless of the year of collection. The liver was the most common habitat of the parasite, and the lung was the most frequent ectopic region, followed by mesentery, pleura, abdominal wall, and kidney. The lesions of the affected organs were generally characterized by well-developed cysts, each containing a larva. However, the cysts within kidney and abdominal wall were poorly organized, filled with necrotized neutrophils, and lacked larvae. Immunohistochemistry for collagen type I, III and IV indicated that collagen type I and III were the major components of the cyst wall. However, their distribution was different depending on the developmental stages; both collagen types contributed equally to cyst formation at the early stage, whereas collagen type I was the major component at the late stage. In early stage cysts, collagen type I was localized in the outer layer while collagen type III was located in the inner layer. In the liver, collagens were likely generated by myofibroblasts that originated from activated hepatic stellate cells, which were predominantly distributed in the outer layers of the cysts. Our results suggest that appropriate timely remodeling process of collagen fibers is necessary for *C. fasciolaris* to build the well-developed cysts in the target organs for survival.

ESTP Poster Abstracts

TP17: Maternal exposure to endocrine disrupting chemicals and health effects on female offspring Sprague Dawley rats

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A range of synthetic as well as naturally occurring agents have been identified as interacting with the endocrine system. If the interaction of these exogenous substances with the endocrine system leads to adverse health effect, these substances are referred to as "endocrine disruptors chemicals" (EDCs). There is growing evidence that EDCs can function at very low doses in a tissue specific manner. The age at which an individual is exposed has important implications on resulting health consequences: exposure to EDCs during early development results in different effects than exposures during adulthood. The accumulation of persistent chemicals classified as EDCs causes an increase in reproductive disorders in males and females and in hormone-dependent cancers. Moreover EDCs have been shown to disrupt normal mammary development and to enhance development of hyperplasia, beaded ducts, or tumors. The aim of the study is to determine whether low doses of Diethylphtalate (DEP), Methylparaben (MPB), Triclosan (TRC), and a mixture of the three EDCs, administered at one dose level starting from Post Natal Day (PND) 1 until PND 180 affect the developmental pattern and the proliferative activity of the mammary gland in adults parous and nulliparous female rats. DEP, MPB, TRC and Mixture solution were administered in olive oil as vehicle by gavage. Based on calibration studies and data from a human case control study, named "Long Island Breast Cancer Study Project (LIBCSP)", the doses selected for the study in mg/Kg/day were: DEP =0,173; MPB =0,105; TRC =0,005. Control group received sham gavage with olive oil alone, which was the selected vehicle. The experiment used female Sprague-Dawley (SD) rats which belong to the colony used in the laboratory of the CMCRC. The females were exposed from PND1 and after divided in two groups: 1) "Parous" female rats mated at 17 weeks of age and exposed until PND 180 of their life; 2) "Nulliparous" female virgin rats exposed until PND 180. Further 3 parous and nulliparous females of each group of treatment, after the weaning of the pups (PND 146 of dams), were selected for macroscopic and microscopic examinations of mammary glands. During the necropsy mammary glands (axillary and inguinal sites) were collected for histopathology examination and whole mount preparations. Compared to average litter mortality of ~3% in oil control group, EDCs treatment resulted in the mortality rates >20% as early as PND7. Treated parous female sacrificed at weaning (PND 146) showed evident histological differences between controls in mammary gland: alveoli were not always milk-filled and an increase of adipose tissue was noted. Whole-mount preparations (WM) confirmed the same morphological pattern. The effect of TRC is particularly evident with much smaller lobuli composed of a lower number of alveoli, often empty. No histopathological differences were observed among treated and control groups in parous females sacrificed at PND 181 where a three-fold reduction of MGs, compared to the lactation, was observed. For the nulliparous females the development of MGs had normal tissue morphology in both treated and control groups. In these virgin females, the duct system was the most prominent component and the lobules of alveoli were sparse and small. Our animal model could be considered a human equivalent model especially for breast cancer allowing us to translate rodent data on mammary gland effects to human. In conclusion, this animal study by mimicking the realistic human exposure to EDCs at very low doses, highlights the heightened sensitivity of the MGs during critical windows of exposure in particular pregnancy and lactation, with impact on pups survival. These results will provide a foundation for future studies in risk assessment for human exposure.

ESTP Poster Abstracts

TP18: Long-term effects of endocrine disruptors: The case of the pesticide Mancozeb

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EU Commission and other international organisations (OECD, WHO) point out as a priority the importance of improving knowledge on "Endocrine Disrupting Chemicals"(EDCs), a heterogeneous group of substances characterised by the potential to interfere, through various mechanisms, with the functioning of the endocrine system, in particular with homeostasis of sex hormones and thyroid.

Many compounds suspected of being endocrine disruptors are substances widely used and with strong economic impact, therefore appropriate solutions for their conscious use are required. Among this wide range of compounds classified as EDC an important role is assumed by pesticides (organophosphates, carbamates and dithio-carbamates, organochlorines, etc). Mancozeb, an ethylene-bis-dithiocarbamate (EBDC), has been one of the most commonly used fungicides in commercial use for several decades. Up to now epidemiological data reported in the literature suggest the carcinogenicity of Mancozeb, but there were no adequate experimental studies confirming the carcinogenic effects of Mancozeb. Because of the importance of the compound and the number of people potentially exposed, a long-term experimental study of Mancozeb was performed at the Cancer Research Center of the Ramazzini Institute and published in 2002.

Groups of 150 male and female Sprague-Dawley rats, 8 weeks old at the start of the treatment, were administered Mancozeb at the concentration of 1000, 500, 100, 10, and 0 ppm in feed supplied *ad libitum* for 104 weeks. At the end of the treatment, animals were kept under controlled conditions until spontaneous death. Mancozeb caused an increase in: total malignant tumors, malignant mammary tumors, follicular carcinomas of the thyroid gland, malignant tumors of the pancreas, Zymbal gland and ear duct carcinomas, hepatocarcinomas, osteosarcomas of the bones of the head, and hemolymphoreticular neoplasias.

In the recent years, in the framework of our project of EDCs,we studied the distribution of the follicular carcinomas by sex and by age. We observed the following: 1) follicular carcinomas were increased by treatment more in females than in males; 2) proliferative pre-neoplastic lesions were also observed in treated animals; 3) interestingly, the statistically significant increase of follicular carcinomas was observed in males and females only after 104 week of treatment. These results confirm the validity of long-term studies, carried out until spontaneous death of the animals: had we sacrificed the animals at 104 weeks of age, this result would never have emerged. On the basis of these data, Mancozeb must be considered a multipotent carcinogenic agent especially for EDC target organs.

ESTP Poster Abstracts

TP19: Stimulatory effect of β-sitosterol on the growth of T47D cells correlates with its ability to activate Estrogen Receptor and CyclinD1 signaling

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The compound β-sitosterol (BS) is one of the most common forms of phytosterols in seeds and fruits of plants and display anti-oxidant, anti-bacterial, anti-inflammatory and anti-cancer effects. However, its effects on the growth of on estrogen-dependent breast cancer cells have not been well documented. In this study,we assessed the influence of BS on mammary tumor growth and the molecular mechanism(s) responsible for.the effects.

Estrogen receptor(ER) positive T47D cells were employed in cell proliferation assay. Cell proliferation was detected using the 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. Western blotting was used to analyse the protein expression of ERα,ERβ and cyclinD1 in cells exposed to BS.ER antagonist 7alpha-[9-(4,4,5,5,5-Pentafluoropentylsulfinyl)nonyl]estra-1,3,5(10)- triene-3,17beta-diol (ICI182780) was employed as a tool in the experiments. Besides, ER negative human embryonic kidney (HEK) 293 cells were employed in luciferase reporter gene assay. The ER subtype specific oestrugenic effect of BS was further demonstrated by luciferase reporter gene assay in HEK 293 cells.

The results show that BS can promote cell proliferation of ER-positive breast cancer cells (T47D) in a dose-dependent manner. However, when ER antagonist (ICI182780) is added together with BS, the promotive effect of BS in cell availability can be inhibited. We also demonstrate that both ERα and ERβ protein levels increase after BS treatment in T47D cells, followed by up-regulation of cyclinD1. However, ER antagonist can suppress the promotive effect of BS towards protein expression after the treatment of ICI182780. Finally; the reporter gene analysis further demonstrates that the action of BS on activating ERE-mediated luciferase transcription is realized *via* ER pathway with a full dose–response, especially through ERβ.

The conclusion is that β-sitosterol can enhance the proliferation of T47D cells, which is obtained by ER-mediated regulation of cyclinD1 signaling pathways. Such finding suggests the widespread use of phytoestrogens could be detrimental, because of their ability to stimulate ER-positive breast cell growth.

ESTP Poster Abstracts

TP20: Mutation of the palmitoylation of estrogen receptor ERα in vivo reveals the crucial role of membrane effects of estrogens in female fertility

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Estrogen Receptor alpha (ERα) activation by Estradiol (E2) leads to direct nuclear genomic actions namely transcriptional activity essential for reproduction, uterine proliferation or metabolism for example However, numerous in vitro data indicate that ERα also interacts with several plasma membrane-associated molecules and thereby elicits membrane-initiated steroid signaling pathways. The physiologic role of this ERα subpopulation in vivo is unknown. Palmitoylation is an important post-translational modification for ERα plasma membrane localization in vitro. We therefore generated a mouse with a point mutation of the palmitoylation site of ERα (C451A-ERα) to obtain membrane-specific loss-of-function of the receptor. The major alteration of membrane localization of ERα in vivo was confirmed in primary hepatocytes as assessed by western blot of plasma membrane fractions Interestingly, the female mutant mice were completely infertile in continuous mating studies, as shown by the absence of litters. To investigate the basis of this infertility, histomorphological analysis was performed on digital slides of ovaries from 10-12 week-old female mice. Microscopic evaluation and counting of the number of follicles at different stages revealed that there was an excess of large, hemorrhagic and/or cystic follicles originating from antral follicles in C451A-ERα ovaries, along with an almost total absence of typical mature corpora lutea. This abrogation of female fertility was associated with disrupted estrous cyclicity, as assessed by vaginal smears. Steroid sex hormone levels were also measured in intact adult female mice. Progesterone production was largely decreased while the luteinizing hormone (LH) was elevated in comparison to wild-type (WT)-ERα mice.

Conclusion: The C451A-ERα mouse demonstrates for the first time the essential role of ERα membrane actions in female fertility. It also offers a new tool to address the physiologic roles of ERα membrane-initiated signaling in vivo in other tissues.

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ESTP Poster Abstracts

TP21: Unexpected pituitary pathology after chronic administration of MEDI412, a high potency Anti-IgE antibody

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Introduction: Elevated serum IgE levels play an important role in certain diseases such as allergic asthma or atopic dermatitis. MEDI4212 binds to cynomolgus monkey, but not rodent, IgE with comparable affinity to human IgE.

Materials and Methods: Three males and three females were treated with weekly SC doses for 26 weeks at 0, 50, or 150 mg/kg. An additional two animals of each gender from each group were then not treated for 13 weeks. A full set of tissues from all animals were fixed in 10% nbf, processed, sectioned, and stained with H&E for evaluation. Additional sections of pituitary gland were also stained using IHC techniques for MEDI4212, FSH, LH, PrL and Ki67.

Results: Hypertrophy of the pituitary gland was seen in all females at 150 mg/kg (high dose) and in 2/3 animals at 50 mg/kg (low dose). This finding was still present at the end of a 13-week recovery period in high dose group only, but no downstream functional effects in endocrine organs were observed. Investigative studies confirmed the absence of hyperplasia and demonstrated that MEDI4212 did not bind to circulating female pituitary hormones or pituitary cells.

Conclusion: Although no clear mechanism of action could be identified in these follow-on studies, a NOAEL of 50 mg/kg once weekly was proposed based on (1) lack of functional consequences, (2) low-grade severity of the lesion and (3) slight trend towards recovery. Off-target effects can occur in the endocrine system with monoclonal antibodies without physiologic consequences for the animal.

ESTP Poster Abstracts

TP22: Gonadal development after photostimulation in Bobwhite quails

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Introduction: A one-Generation Reproduction Study was performed to evaluate the influence of photostimulation (daylight) on the development of testes and ovaries in the Bobwhite quail (Colinus virginianus).

Materials and Methods: 38 male and 38 female Bobwhite quails were obtained from a commercial breeder. After arrival, the animals were identified by leg bands, weighed and, at an age of 17 weeks, randomized on the basis of body weight into two groups

(E and F, differing in their respective diets). Breeding pairs were housed in stainless steel cages for up to 24 weeks. The first 7 weeks, the daylight period was 7 hours/day, in weeks 8 and 9 it was increased to 14 hours/day and from week 10 onward to 17 hours. From both groups (E and F) 3 pairs each were sacrificed at the beginning of the study, one pair each was sacrificed after week 7, 8, 9, 10, 11, 12, and 16, respectively, 5 pairs each were sacrificed after 20 weeks, and 4 pairs each were sacrificed after 24 weeks. The birds were examined macroscopically, left and right testis (including epididymis) and ovaries were weighed, fixed in modified Davidson's solution, processed histotechnically, stained with hematoxylin and eosin, and examined histopathologically.

Results: The testes weights remained comparable between start of the study up to week 8 and between the two test groups E and F. Starting after week 9, a steep weight increase was observed up to week 16. Afterwards up to week 24, the testes weights slightly decreased. The weight development was nearly comparable between the left and the right testis, with the weight of the right testis being minimally lower than that of the left testis.

The ovarian weights were comparable between start of the study up to week 9. A slight increase was observed up to week 11, followed by a steep increase until week 24.

The development of testes and ovaries could be easily seen macroscopically.

Histologically, the maturation of testes starts about week 9 (1 week after increase of the photoperiod), and was finished between week 11 to 12.

The histopathological examination of ovaries was less useful as histopathology reflected the increase in egg yolk which was already seen on macroscopical investigation.

Impact statement: The description of the normal development of the Bobwhite quail gonads during the various phases of photostimulation facilitates the interpretation of histological findings in future reproduction studies, in which usually only data from one specific time point will be available.

ESTP Poster Abstracts

TP23: High animal-fat intake enhances prostate cancer progression and reduces glutathione peroxidase 3 expression in early stages of TRAMP Mice

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BACKGROUND: Prostate cancer is the most frequently diagnosed cancer in Western men, and more men have been diagnosed at younger ages in recent years. A high-fat Western-style diet is a known risk factor for prostate cancer and increases oxidative stress.

METHODS: We evaluated the association between dietary animal fat and expression of antioxidant enzymes, particularly glutathione peroxidase 3 (GPx3), in the early stages of transgenic adenocarcinoma of the mouse prostate (TRAMP) mice. Six-week-old male nontransgenic and TRAMP mice (Jackson Laboratory, Bar Harbor, ME, USA) were placed on high animal-fat (45% Kcal fat) or control (10% Kcal fat) diets and sacrificed after 5 or 10 weeks. We evaluated histopathologically each lobe of the prostate (ventral, dorsal, lateral, and anterior) and assigned a prostate intraepithelial neoplasia (PIN) grade as PIN I (relative small foci with one or two layers of atypical cells), PIN II (larger foci with two or more layers of atypical cells that do not fill the lumen), PIN III (the foci of atypical cells fill, or almost fill, the lumen of the ducts), or PIN IV (the foci of atypical cells fill the lumen of the ducts), based on the most severe lesion within the lobe. The histopathological score was determined by calculating a distribution (focal, multifocal, or diffuse)-adjusted PIN score (o to 12). PC-3 human prostate cancer cell lines were treated with cholesterol and cell proliferation was measured by MTT assay. GPx3 mRNA and protein levels were evaluated by real time RT-PCR and Western blot analysis, respectively.

RESULTS: The histopathological score increased with age and high-fat diet consumption. The histopathological scores in dorsal and lateral lobes increased in the 10-week high-fat diet group (6.2 ± 0.2 and 6.2 ± 0.4, respectively) vs. the 10-week control diet group (5.3 ± 0.3 and 5.2 ± 0.2, respectively). GPx3 decreased both at the mRNA and protein levels in mouse prostate. GPx3 mRNA expression decreased (~36.27% and ~23.91%, respectively) in the anterior and dorsolateral prostate of TRAMP mice fed a high-fat diet compared to TRAMP mice fed a control diet. Cholesterol treatment increased PC-3 human prostate cancer cell proliferation, decreased GPx3 mRNA and protein levels, and increased H_2O_2 levels in culture medium. Moreover, increasing GPx3 mRNA expression by troglitazone in PC-3 cells decreased cell proliferation and lowered H_2O_2 levels.

CONCLUSIONS: Dietary fat enhances prostate cancer progression, possibly by suppressing GPx3 expression and increasing occurrence of prostate intraepithelial neoplasia (PIN) in epithelial cells.

ESTP Poster Abstracts

TP24: Mass Spectrometry Imaging in a toxicology study: Application in induced interstitial pulmonary fibrosis (IPF) model

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The identification of the potential causes of the histopathologic/functional tissue changes is one of the main difficulties in toxicology studies. Mass spectrometry imaging (MSI) technology has been used to address these crucial issues. The main advantage of this label free imaging technique is the detection of all molecules of interest directly on-tissues with high specificity. Indeed the molecular distribution of some unlabeled targeted molecules could be directly correlated with some histopathologic and functional tissue changes. MSI was used to improve the understanding of the Bleomycin-induced interstitial pulmonary fibrosis rat model. Rats were administered seven doses of bleomycin delivered to the lungs and followed for 14 days. Control animals received seven doses of saline. Control and treated animals were sacrificed and lungs were collected. Several fresh sections were prepared and then molecular imaging was performed by high spectral resolution and high spatial resolution-MSI. Based on the combination of MSI and staining, lysophosphatidic acids (LPAs) were detected and confirmed to be specifically distributed in the fibrosis area. A significant difference of signal was observed for LPAs in the IPF tissues compared to the control tissues. LPA was described in the literature to contribute to the development of fibrosis after lung injury through multiple mechanisms (via LPA_1 and LPA_2 receptors). Moreover, some other specific ions of the fibrosis are currently being identified as well as some other potential markers at a higher mass range (>1000 Da). MSI provides the identification of markers/readouts in toxicology studies associated with atypical toxicologic pathology findings.

ESTP Poster Abstracts

TP25: Assessment of drug toxicity in small histological structure of the eye: Application of Mass Spectrometry Imaging in ophthalmic context.

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The eye is a highly complex tissue which consists of several small organs or structures. It can be basically divided into two regions, the anterior and the posterior parts, including the most important ocular structures. The anterior part consists of cornea, iridocorneal angle, trabecular meshwork, iris lens and aqueous humor. The posterior part contains the vitreous humor and the retina region (retina, sclera and choroid layers and the optic nerve, which is an extension of central nervous system). The two major eye diseases, Glaucoma and the Age-related Macular Degeneration (AMD), are closely correlated with these two histological regions of the eye. Mass spectrometry Imaging (MSI) applications to ophthalmic drug discovery have recently gained growing interest especially for preclinical studies in pharmacology or toxicology. In our study, MSI was applied to assess the distribution and quantification of Benzalkonium chloride (BAK) compound (antiglaucoma eye drops preservative) in specific areas of the eye after instillation. The distribution of two BAK compounds (BAK C12 and BAK C14) was investigated in small specific histological regions of the eye (such as iridocorneal angle or sclera, choroid, retina regions) in order to estimate efficiency of action or adverse effects of the treatment. High spatial resolution images were performed at cells level (30 µm). Molecular distribution was also correlated to tissue histology using H&E staining or IHC. Local Drug concentration differences were observed according to histological area and position on the eye section (anterior, posterior, temporal or nasal side). In conclusion, MSI offers new insight in ocular therapeutic/pharmaceutical research, especially to give a better understanding of the drug candidate migration from the front to the back of the eye to assist drug efficiency or toxicity studies for specific tissue targeting eye diseases.

ESTP Poster Abstracts

TP26: Significant lessening of local reactions following continuous subcutaneous administration of ND0701, a new apomorphine formulation for Parkinson's Disease – MRI and histopathology studies

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Introduction: Subcutaneous administration of Apomorphin is suggested as a replacement therapy for Levodopa in Parkinson's disease patients. Continuous subcutaneous infusion of drug formulations often results in local damage at the administration site. *In* vivo and *ex vivo* MRI was used to evaluate the local damage of commercial Apo-Go® and a newly developed apomorphine formulation ND070 in live animals and tissue biopsies. The results were compared to histopathology.

Experimental Design & Methods: The drug formulations ND0701 and Apo-Go® were administered to domestic pigs by 24 hour continuous subcutaneous infusion using an infusion pump. Follow-up of damage at the infusion site was performed using *in vivo* MRI, two and four weeks post drug administration in a Magnetom-C MRI machine (Siemens). *Ex vivo* MRI was performed thereafter on excised formalin-fixed skin tissues, using the novel compact 3D-MR based histology system (Aspect Imaging) followed by histopathology. The histological evaluation consisted of a subjective description of the observed tissue reaction and was scored according to its severity.

Results: *In vivo* MRI showed significantly smaller lesions at the infusion site 2 weeks post infusion, with almost no damage observed after 4 weeks following 1% ND0701 administration. Damage was still clearly evident after 4 weeks following 1% Apo-Go® administration. *Ex vivo* compact MRI and histopathology revealed severe damage following Apo-Go® injection, consisting of necrosis surrounded by granulomatous inflammation, whereas minor chronic inflammation was observed for 1% ND0701.

Conclusion: While *in vivo* MRI was highly efficient in following the recovery at the injection sites, *ex vivo* MRI allowed quantification of the damage with good correlation to histopathology. ND0701 was proved to be better locally tolerated than Apo-Go®.

Impact statement: Based on our experiments, MRI was proven useful in evaluating local toxicologic damage as a result of subcutaneous drug administrations.

ESTP Poster Abstracts

TP27: Automated *in situ* hybridisation with the RNAscope®VS assay for use in drug discovery and development

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Immunohistochemistry (IHC) and *in situ* hybridisation (ISH) are currently the most commonly used methods for the detection of proteins and nucleic acids in tissue sections and both methods can complement each other. While IHC is established in most laboratories, ISH is regarded as a time-consuming, labor-intensive and technically complex method. Depending on the type of the probe (oligonucleotide or riboprobe) the optimization of this method can be difficult.

In order to overcome this hurdle, Ventana Medical Systems Inc. (a member of the Roche group) in collaboration with Advanced Cell Diagnostics Inc. (ACD) launched a fully automated mRNA detection system. The RNAscope®VS assay is designed for use with the Ventana Discovery Ultra® and Discovery XT® automated tissue staining systems based on ACD's patented signal amplification and background suppression technology to visualize single RNA molecules per cell in FFPE samples mounted on slides.

The assay is fully automated, highly reproducible and consistent from run to run providing more accurate and reliable data. Full automation liberates lab resources, while at the same time providing high quality staining results and very good tissue morphology suitable for automated image analysis.

In our laboratory, we have successfully established an automated ISH in discovery and development:

- to complement IHC in order to confirm results, e.g. alpha-synuclein (Snca), erythropoietin receptor (Epor), endothelial-specific receptor tyrosine kinase (Tek)
- as a supplementary method where IHC fails (no antibodies suitable for IHC, i.e. for mouse and rat or high unspecific background staining), e.g. discoidin domain receptor family member 1 (Ddr1), ectonucleotide pyrophosphatase/phosphodiesterase 2 (Enpp2), glucagon-like peptide 1 receptor (Glp1r), hepcidin antimicrobial peptide (Hamp)
- for proteins with very low expression/translation (target expression and distribution), e.g. Ddr1, Tek
- in combination with IHC (double stain) to show co-localisation, e.g. growth differentiation factor 15 (Gdf15) with CD68 IHC.

So far, there are only a few reports available for the fully automated RNAscope®VS assay, mainly related to human samples in virology and oncology. To our knowledge, this is the first report describing this application as an additional routine tool in drug discovery and development complementing IHC.

ESTP Poster Abstracts

TP28: *In situ* characterisation of murine models of dendritic cell ablation and altered function

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Dendritic cells (DC) are "professional" antigen-presenting cells which play a pivotal role in regulating innate and adaptive immunity. DC sample antigens in the local microenvironment and present them on their surface alongside appropriate costimulatory molecules. Antigen presentation by DC results in T cell activation, the best-known function of DC, but also, for example, induction and preservation of immune tolerance, stimulation of B cells and maintenance of B cell memory, all less characterised aspects of DC physiology. Due to their role in initiating and coordinating the immune response, which requires interaction with several different immune cell types, DC express numerous cell surface markers. However, none of these allows specific identification of DC. The lack of an antigen exclusively expressed by DC, coupled with the growing evidence that DC consist of several phenotypically distinct populations rather than being part of a homogeneous family, has complicated investigations on their development and function.

Depletion of DC *in vivo* or knockout (KO) of genes specifically expressed by DC or involved in their development are examples of the most recent and powerful approaches applied to explore DC function.

Here, we present a histological and immunohistological workup of selected mouse models of DC depletion or altered function. Our preliminary analysis includes morphological comparison between C57BL/6 wild type mice and the following models: 1) C57BL/6 mice treated with liposomal chlodronate (selective uptake by and depletion of macrophages and DC); 2) C57BL/6 mice engineered to express human diphtheria toxin receptor (DTR), treated with a murine *cd11c* promoter to drive transgenic expression of DTR in DC and administered diphtheria toxin; 3) C57BL/6 mice KO for tyrosine kinase receptor FMS-like 3 (Flt3), required for DC development in peripheral lymphoid tissues; and 4) C57BL/6 mice KO for Batf3, an activator protein 1 (AP-1) transcriptor factor needed for the development of specific subsets of DC. Mice underwent a complete histological phenotypic analysis, followed by immunohistological characterisation of the lymphoid tissues using markers for T and B lymphocytes, macrophages and DC (not exclusive for DC).

Each model exhibited peculiar histopathological changes (e.g. macrophage depletion in the spleen in chlodronate-treated mice and dendritic cell apoptosis in DTR-DT mice), together with alterations in the distribution and number of specific subsets of lymphocytes, macrophages and DC.

Recognition of DC and DC subsets is routinely carried out using flow cytometry, which does not provide information on architectural relationship between the examined cells and their surroundings. Morphological characterisation of *in vivo* models targeting DC may bring new insight into the understanding of the role of DC and, in particular, can help researchers interested in this field to understand the advantages and limitations that should be taken into consideration when interpreting results obtained with mouse models of DC depletion/altered function.

ESTP Poster Abstracts

TP29: MicroRNA profiling in a mouse model of drug-induced kidney injury

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Acute kidney injury (AKI) is a major worldwide health issue, particularly in the developed western world. Drug-induced kidney injury (DIKI) accounts for a large proportion (estimated 19%) of AKI acquired during periods of hospitalisation.

The current "gold standard" biomarkers of AKI in man and animals are serum creatinine and blood urea nitrogen, both of which have limitations regarding their pre-clinical and clinical use and require drawing blood. Micro RNAs (miRNAs) are short non-coding RNA sequences (18-25 nucleotides in length) that are highly conserved throughout evolution and are very stable in serum and urine.

Using the anti-cancer agent cisplatin with its well characterised nephrotoxic effect, we established a mouse model of AKI and profiled a total of 394 miRNA species in urine, serum and kidneys in order to investigate the potential of miRNAs as biomarkers of AKI, with the expectation that they outperform current "gold standards" in terms of sensitivity.

Male CD1 mice (28-35 g) were administered intraperitoneally in groups of 4 animals a dose of 20mg/kg cisplatin or 0.9% saline (controls) and were sacrificed 24, 48, 72 and 96 h post dosing (hpd), after 24 h housing in individual metabolic cages. Urine served to quantify urinary creatinine (UCr) and N-acetyl-β-glucosaminidase (NAG). Blood was collected via cardiac puncture and blood urea nitrogen (BUN) was determined. Kidneys were removed for histological and immunohistological (cleaved caspase-3) examination as well as miRNA isolation (48 h group). After miRNA purification, cDNA preparation and pre-amplification, miRNA arrays (Megaplex Rodent Primer Pool sets) were run on serum, urine and kidneys and the analysis undertaken with Expression Suite software (both Applied Biosystems).

Histology provided first evidence of AKI at 24 hpd when scattered individual apoptotic proximal tubular epithelial cells were observed. The extent of tubular epithelial cell apoptosis increased with time and was accompanied by progressive protein cast formation.

Among biomarkers, UCr was not significantly elevated at any time point and thereby served as a normalising tool. Urine NAG and BUN were significantly higher than in controls in the 72 h and the 96 h group respectively. The miRNA analysis, undertaken in the 48 h group when the conventional biomarkers were not yet elevated, identified a range of significantly altered miRNA species. In urine, 18 miRNAs were increased by more than two fold, with 4 being particularly high (11.8 to 27 fold), i.e. miR15a (targets bcl-2), 744 (enhances cell proliferation), 365 and let-7e (highly expressed in the kidney). In the kidney, the increase in MiR-205 (involved in epithelial to mesenchymal cell transition) and MiR-34a (involved in the apoptotic response), and in the serum, miRNAs involved in epithelial to mesenchymal cell transition, were particularly noteworthy.

Histology in combination with the conventional biomarkers confirmed that intraperitoneal administration of cisplatin induces significant AKI in mice, at a dose of 20mg/kg. Our investigation shows that several miRNA species are significantly upregulated early after dosing, at a time, when conventional biomarkers are not yet elevated, and also in the urine. Most of these miRNAs reflect the renal damage, and in particular the mode of cell death, apoptosis, in mice after cisplatin treatment. This suggests that urinary miRNA species may have potential as biomarkers of DIKI.

ESTP Poster Abstracts

TP30: New biomarkers as a valuable tool for enhanced histopathology in preclinical immunotoxicity studies

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According to the OECD 407 guideline (revised in 1995) an advanced screening battery was introduced to detect immunotoxic findings in preclinical studies. Histopathology of lymphoid organs based on H&E-stained paraffin sections is one major part of immunotoxicological evaluation. Other methods are organ weights, analysis of subpopulations and functional testing of immune cells. Due to the complexity of immune system there is no single detection method for an immunotoxic substance.

Here we present a new method combining enhanced histopathology and a panel of readily available immunohistochemical markers for use in paraffin-embedded material in OECD 407 studies. Enhanced histopathology is a validated tool that the pathologist can use to examine lymphoid organs per compartment to achieve higher diagnostic sensitivity. It applies a terminology which is descriptive rather than interpretative and evaluates all changes including those which are often seen as "normal background". The introduction of biomarkers such as immunohistochemical antibodies to quantify subpopulations of immune cells or changes such as apoptosis is a new approach in this context. Technically the belated use of immunohistochemical markers can be challenging in materials from toxicological studies due to prolonged fixation or storage times.

In a 28-day validation study in 28 male Wistar rats (7 – 8 weeks old at start of the study; Harlan, Horst, the Netherlands) the immunosuppressive drug Azathioprine was administered orally at dose levels up to 20 mg/kg b.w. per day. Six different antibodies were applied in animals dosed at 0 or 20 mg/kg to paraffin sections of thymus, spleen and mesenteric lymph node to identify the amount and distribution of T cells (CD3- and CD8-positive), B cells (CD79-positive), macrophages (CD68) and epithelial cells (pan cytokeratin) as well as apoptosis (caspase-3). The semi-quantitative evaluation resulted in a difference mainly in thymus and spleen (e.g. in cell counts, distribution of cells and structural organisation within the organs) between control and treated animals which was not obvious in the conventional H&E stain.

Furthermore a quantitative evaluation of other immunotoxic studies by immunohistochemical evaluation and image analysis is planned to detect morphological differences not obvious in semi-quantitative evaluation of H&E stains. Further plans are the comparison of the results to immunological test such as FACS and functional tests.

In conclusion, evaluation of immunohistochemical biomarkers yielded additional information in this investigation which could not be obtained in H&E-stained sections. The use of immunohistochemical markers thus adds value to routine guideline studies giving more insight into immunomodulatory effects. This approach can be useful for mechanistic investigations during preclinical development of pharmaceuticals and risk assessment of chemicals.

ESTP Poster Abstracts

TP31: Early time response of liver cells to facilitate cell cycle aberration by treatment with hepatocarcinogen in rats

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Background: We have previously reported that carcinogens evoking high proliferation activity to carcinogenic target cells increased populations expressing cell cycle-related molecules, especially of G_2/M or M phase, and facilitated apoptosis after 28-day treatment in rats. Carcinogen-treatment also induced aberrant expression of ubiquitin D (Ubd), a M-phase progression-related molecule, from G_2 phase, suggestive of cell cycle aberration involving both G_2/M and spindle checkpoints.

Aim: The present study was performed to determine the time response of liver cells to undergo carcinogen-specific facilitation of cell cycle aberration and proliferation after hepatocarcinogen-treatment for up to 28 days. For comparison, cellular responses after partial hepatectomy (PH) or treatment with non-carcinogenic hepatotoxicants were examined.

Materials and Methods: Male 6-week-old F344 rats were divided into groups of untreated controls, PH, hepatocarcinogen-treatment [thioacetamide (TAA) and methyleugenol (MEG)], and non-carcinogenic hepatotoxicant-treatment [α-naphtyl isothiocyanate (ANIT), acetaminophen (APAP) and promethazine hydrochloride (PMZ)]. Liver tissues were immunohistochemically examined at days 3, 7 and 28 after the start of treatment.

Results: At day 3, PH, TAA and ANIT increased liver cell proliferation and cells expressing G_2/M or M phase molecules. TAA and ANIT also increased the Ubd^+ cells within $TopoII\alpha^+$ cell population by analysis using double immunohistochemistry, but PH did not. At day 7 of treatment, PH, MEG, TAA, APAP and ANIT decreased or did not increase liver cell proliferation and cells expressing G_2/M or M phase molecules, and MEG, TAA, APAP and ANIT increased or tended to increase cells expressing $p21^{Cip1}$ and frequency of apoptosis. At day 28, MEG, TAA and PMZ increased liver cell proliferation and cells expressing G_2/M or M phase molecules, and MEG and TAA specifically increased cells expressing $p21^{Cip1}$ and frequency of apoptosis, but PMZ did not. APAP also increased $p21^{Cip1+}$ cells. PH, APAP and ANIT did not increase cell proliferation, cells expressing G_2/M or M phase molecules and frequency of apoptosis. In addition, MEG and TAA decreased p-Histone $H3^+$/ $Ki\text{-}67^+$ cell ratio as compared with untreated controls and PMZ. MEG and TAA also decreased the p-Histone $H3^+$ cells within Ubd^+ cell population and increased the Ubd^+ cells within $TopoII\alpha^+$ cells population, but PMZ did not.

Discussion: The present study revealed that increase of cells expressing G_2/M or M phase molecules was in parallel with cell proliferation activity at day 3. Increase of Ubd^+ cells at G_2 phase was suggested to be the reflection of cell proliferation as a result of cellular toxicity. On day 7, cells activating G_1/S checkpoint or undergoing apoptosis were increased without accompanying activation of cell proliferation probably in response to toxicity of chemicals irrespective of their carcinogenic potential. On day 28 of treatment, hepatocarcinogens specifically increased cell proliferation activity and apoptosis, associated with activation of $p21^{Cip1}$, G_2/M arrest, aberrant expression of Ubd from G_2 phase, and decreased ratio of proliferating cells at M phase. These results suggest that carcinogen-specific responses of simultaneous cell cycle facilitation and Ubd dysfunction to cause spindle checkpoint disruption can be detected at least at 28-day of treatment. Induction of apoptosis may be due to the activation of G_1/S and G_2/M checkpoint functions.

ESTP Poster Abstracts

TP32: Histopathological assessement of induced toxicity in three-dimensional (3D) Liver Spheroids

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3D cell culture models have been developed for a variety of tissues under different conditions in vitro, supported by 3-dimensional scaffold structures or free of any scaffold. Under such conditions, the disadvantages of 2-dimensional systems may be avoided, e.g hepatic 2D- systems often fail to predict toxicity induced by different classes of chemical compounds. The latter is especially true for hepatotoxicity due to changes in cell–cell contacts, polarisation, loss of metabolizing enzymes, etc.

The establishment of such 3D-hepatocellular models has been published, whereby such micro-organs are not necessarily composed by hepatocytes only but they may be co-cultured with a range of other cells important for metabolic functions, e.g. stellate cells, Kupffer's cells etc. The model functionality was proven by positive control compounds. Furthermore, these 3D-multi-cell type liver micro tissue represents a stable and functionally active model system, with sustained expression for more than three weeks of cultivation of important metabolic proteins regulated by the glucocorticoid and anti-oxidant cell defense pathway.

Usually, enzyme activity, metabolism and gene expression analytics are endpoints in testing of chemical compounds in spheroid organs. Histopathology evaluation was supposed to be supportive getting a better understanding of induced toxicities. Furthermore, adaptations of such a classical method were deemed to be predictive linkers between other evaluation endpoints. Therefore, a number of positive compounds known of different mechanistics for the induction of liver injury where tested on hepatocellular 3D models (liver spheroids). The tissues underwent histological processing and were prepared for paraffin and plastic sections. The evaluation was performed by classical methods (e.g. hematoxylin and eosin, PAS, Masson's trichrom), immunohistochemistry including sophisticated methods, such as laser scan etc.

ESTP Poster Abstracts

TP33: Preliminary CEACAM5 protein and mRNA expression in hCEA-hCD3ε transgenic mice

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Carcinoembryonic antigen (CEA), also called carcinoembryonic antigen related cell adhesion molecule 5 (CEACAM5), is a glycosylphosphatidylinisotol (GPI) anchored cell-surface glycoprotein (180- 200 kDa). CEA mainly serves as a cell adhesion molecule mediating intercellular contact in normal tissues, whilst in cancer it contributes to tumor invasion and metastasis via binding to selectins. In human tumor tissue CEA is highly overexpressed in most gastro-intestinal malignancies and in lung, pancreatic, breast and thyroid cancers. In the healthy adult, CEA expression is restricted primarily to glandular epithelial cells along the gastro intestinal tract, columnar epithelial cells and goblet cells in colon, mucous neck cells and pyloric mucous cells in the stomach, squamous epithelial cells of the tongue, esophagus and cervix, secretory epithelia and duct cells of sweat glands, and epithelial cells of the prostate.

To provide a preclinical model for immunotherapy, mice transgenic for human CEACAM5 and human CD3ε were generated by crossing C57BL/6J-TgN(CE4Ge)18FJP mice (Clarke et al. 1998) with huCD3ε mice. The aim of the present investigation was to evaluate the CEACAM5 protein and mRNA tissue expression profiles in these double-transgenic mice.

Immunohistochemistry (IHC) was performed on a full set of fresh frozen OCT-embedded tissues collected at necropsy from two transgenic mice/sex using an anti-human CEA IgG antibody (T84.66sf W(1a)) specific for CEACAM5 cloned with rabbit Fc (Roche Glycart). Tissue expression analysis was performed on a subset of RNA-later preserved tissues from three additional transgenic mice with RT-qPCR assays specific for human CEACAM5 and in parallel on a subset of human tissues.

Positive staining for CEACAM5 protein in hCEA-hCD3ε transgenic mice tissues was observed using IHC in the colon, with moderate to strong staining of the enterocytes in the apical portion of the crypts. Approximately less than 10% of enterocytes in the whole colon sections were positive. Weak staining was noted in rare cells with focal distribution in the stomach, cecum and cerebellum. All other examined tissues did not exhibit any staining.

Human CEACAM5 mRNA was expressed in colon, stomach, trachea, brain and cerebellum in hCEA-hCD3ε transgenic mice. These data suggest a good correlation between mRNA expression and CEA protein distribution assessed by IHC in the different organs investigated. Similar mRNA expression was noted in human colon, stomach and trachea, while no mRNA expression was present in human brain and cerebellum. In addition, CEACAM5 mRNA signals were detected in lung of hCEA-hCD3ε transgenic mice at very low expression levels, much lower than the signals in lung tissue from the human donor.

In summary, the CEACAM5 protein and mRNA tissue expression profiles in this hCEA-hCD3ε transgenic mouse model show differences in pattern, distribution and extent to what is reported for normal human tissues. In particular the results obtained suggest a low level of human CEA expression in the gastro-intestinal tract in the hCEA-hCD3ε transgenic mouse model in comparison to humans. Conversely, presence of CEACAM5 in the brain tissue seems to be a feature limited to this double-transgenic mouse model. These ex vivo analyses suggest that this hCEA/hCD3e double transgenic mouse model is of limited use for the safety assessment of drugs targeting hCEA since its expression do not fully recapitulate the human pattern.

ESTP Poster Abstracts

TP34: L1CAM Protein expression of selected tissues from humans and non-human primates

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Introduction: The L1 cell adhesion molecule (L1CAM) is a transmembrane protein important for the development of the nervous system, normally expressed in neurons and kidney tubules in man. Increased L1CAM protein expression is reported for various types of cancer. In order to assess L1CAM as a potential target for cancer therapy and the relevance of non-human primates (NHP) as a safety model, we investigated L1CAM protein expression in normal tissues from NHP and humans.

Materials and Methods: Cynomolgus macaque and human-derived formalin-fixed, paraffin-embedded tissue microarrays (TMA) and cynomolgus macaque whole tissue sections were stained for HE and IHC using an L1CAM monoclonal antibody.

Results L1CAM was strongly expressed in neuropil of gray matter in cerebrum and cerebellum, cytoplasm of Purkinje cells and peripheral nerves, and membranes of distal tubules and collecting ducts of kidneys from human and NHP. Moderate cytoplasmic expression was observed in epithelial cells of the gastrointestinal tract, skeletal myocytes, glandular epithelial cells and endothelial cells from both species. In most cases, L1CAM staining in NHP TMAs correlated well with full tissue sections, although differences in staining intensity were observed.

Discussion: Protein expression of L1CAM was identified in most of the examined tissues, and there was good concordance between human and NHP tissue expression. Therefore, the NHP is an appropriate animal model for safety assessment of L1CAM-targeted therapies. The protein expression profile is a useful indication for possible safety concerns. TMAs have an advantage over full tissue sections for early screening of multiple tissue samples from different donors, although effects on staining intensity may be observed.

ESTP Poster Abstracts

TP35: Metabolic shift in cancer cells – insights from an acute leukemia xenotransplantation model

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Cancers evolve by a reiterative process of clonal expansion, genetic diversification and clonal selection within the adaptive landscapes of tissue ecosystems. This diversity entails also phenotypic features that include metabolic drifts in cancer cells. Strong microenvironmental selective forces (and therapeutic interventions) may therefore decimate cancer clones, and erode their habitats, and provide potent selective pressure for the expansion of resistant variants.

In an effort to establish a methodological framework for analysis of metabolites and metabolic pathways in this complex process, we have developed an experimental system to further dissect this process.

By selectively isolating tumor cells form the Central Nervous System and Bone Marrow, in an orthotopic xenograft model of acute myeloid leukemia (AML, HEL cell line, intra-tibia injection), we have derived several pairs of AML cell lines (CNS-AML and BM-AML) that originate from the same parental cell line but have colonized/invaded different organs.

Detailed in vivo serial transplantations assays, histological and electron microscopy analysis, ex-vivo 1H NMR spectroscopy, and RNA and protein expression profiles were used to compare the metabolic profile of these cells lines.

Our results show that both systemic and therapeutic pressures are associated with metabolic diversification and selection of specific cancer variants.

ESTP Poster Abstracts

TP36: Downregulation of autophagic molecules inhibits chondrocyte senescence in hypoxic exposed nucleus pulposus

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Background: Degeneration of intervertebral disc (IVD) is the common cause of low back pain, leading to low quality of life and high economic cost. The main features of the degenerated IVD are the degradation of nucleus pulposus (NP) cells viability and the loss of matrix components such as type IIcollagen, aggrecan.

Currently, a large number of studies suggest that programmed cell death (PCD) plays a central role in development of IVD degeneration. In addition, senescence and autophagy are thought to play key mechanistic role in development of multiple ageing-associated diseases.

Purpose: Thus, the current study is designed to evaluate effect of matrix components and death of cultured NP under serial culture passage and hypoxic condition. This innovative model allows to determine IVD microenvironment components in types of cell death and to investigate IVD tissue engineering by stem cell therapy and molecular therapy using highly biocompatible biomaterials.

Methods: NP cells were isolated from lumbar discs of SD rats. The cells of serial passage cultured at 37 °C under 5% CO_2 or in a hypoxia chamber at 5% O_2 were collected and examined using real-time polymerase chain reaction (PCR), monodansylcadaverine (MDC) staining and Alizarin Red S staining. The level changes of gene expression were analysed for chondrogenic molecular, autophagic and apoptotic gene marker.

Results: There was no significant difference between normal condition and hypoxic condition in morphometric analysis of serial passage cultured NP cells. NP cells underwent apoptosis under normal condition by upregulation of caspase-3 and caspase-8. However, the NP cells in hypoxic condition were detected at a low level of basal autophagy, as Beclin-1, HMGB-1, and LC3-II concomitantly with decrease of p21. Moreover, we found significantly decreased level of pro-apoptotic Bax, caspase-3, caspase-8 and modulation between p53 and Bcl-2 by culture passages. Interestingly, we found the greater expressions of aggrecan in hypoxic group, while the most marked changes in type I, III collagen, ALP and BMP2 mRNA in normal condition.

Conclusions: The present study elucidated that hypoxic condition in NP cells is decreased apoptosis compared to normal condition and that downregulation of autophagic-related molecules may be crucial survival of NP through inhibition of senescence and regulation of ECM.

ESTP Poster Abstracts

TP37: The neonatal piglet as a research model for infant formula safety assessments: Clinical and pathological endpoints in control neonates

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Although breast milk is generally considered to be the optimal nutritional source for human infants during the first year of life, infant formulas have offered an alternative in those instances for which breast milk is unavailable or inadequate for proper nutritional support. There has been a great interest in investigating potential new ingredients for infant formula, including many constituents of human breast milk. The neonatal piglet offers an excellent animal research model for specialised preclinical testing given the need to evaluate these ingredients for safety in an appropriate context. In comparison to the more commonly used rodent models, the piglet offers a closer comparator to the human infant based on its size, similar physiology of digestion, and composition of the maternal milk. In addition, pigs are the only species that can readily tolerate postnatal separation and adapt to nutritional support without maternal influence. This animal model uses 2-day-old domestic Yorkshire-Crossbred piglets that are offered a milk replacer formula as the vehicle control to which new ingredients are then added and tested for the three week lactation period of swine. The aim of this presentation is to provide insight into the significance of the typical growth, clinical pathology parameters, and common macroscopic and microscopic findings in pre-weaned formula-fed piglets, as well as to highlight the differences from adult pigs.

Neonatal animals exhibit several significant differences among clinical pathology endpoints as compared to adults. Microscopic anatomy of the gastrointestinal system of neonatal piglets differs from adult pigs, and may complicate histopathological evaluation due to the developing nature of the immature intestinal tract. It is important to be familiar with the unique physiologic and hematologic profiles, as well as common histological background findings in young growing piglets in order to accurately identify and interpret any relevant effects on these endpoints.

ESTP Poster Abstracts

TP38: Lack of oral toxicity and *in vivo* genotoxicity of the naturally occurring flavonoid, Myricitrin

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Introduction: Chinese bayberry (*Mirica rubra* SIEBOLD) extract (myricitrin) is used as a food additive and antioxidant in flavor modifiers, snack foods, dairy products, and beverages in Japan. Myricitrin is affirmed as "generally recognised as safe" by the U.S. Flavor and Extract Manufacturer Association. The genotoxic and toxic potential of myricitrin was evaluated in anticipation of a positive safety opinion from JECFA (Joint FAO/WHO Expert Committee on Food Additives), and the eventual global marketing of products containing myricitrin.

Experimental Design: Myricitrin was evaluated in a bacterial reverse mutation assay, an *in vitro* micronucleus assay using human TK6 lymphoblast cells, and a 3-day combined micronucleus (peripheral blood) and Comet (liver and duodenum) assay in male and female B6C3F1 mice. Myricitrin was also evaluated in a 90-day toxicity study using male and female Sprague Dawley rats. All studies were conducted according to OECD testing guidelines.

Results: Negative results were observed for myricitrin in both the bacterial mutation and *in vitro* micronucleus assays. No induction of micronuclei or DNA damage was observed in mice following exposure to myricitrin. No adverse clinical pathology or histopathologic changes were present in rats in the repeat dose study using myricitrin at dietary concentrations up to 5% (2926 mg/kg/day for males and 3170 mg/kg/day for females).

Conclusion: Neither *in vivo* genotoxicity studies nor a 13-week dietary study provide evidence of genotoxic potential or toxicity of myricitrin, supporting its safe use in food and beverages.

ESTP Poster Abstracts

TP39: Antioxidant and antimutagenic activities of apple juice concentrate in blood and liver of rats exposed to cadmium

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AIM: The aim of this study was to evaluate the antimutagenic, antigenotoxic and antioxidant activities of apple juice in rats subjected to intoxication with cadmium chloride.

MATERIAL AND METHODS: A total of fifteen male Wistar rats from Centro de Desenvolvimento de Modelos Experimentais (CEDEME – Federal University of São Paulo, SP, Brazil) were distributed in three groups (n=5), as follows: Control group (non-treated group, CTRL), Cadmium group (Cd) and Cadmium-Apple Juice group (Cd+AJ), which received intraperitoneal injection of cadmium chloride (1.2 mg/kg body weight) diluted in water. Additionally, after 15 days, Cd+AJ received apple juice concentrate for 15 days, by gavage at 1.18 g/kg/day.

RESULTS: Apple juice was able to decrease genotoxic effects induced by cadmium in peripheral blood and liver cells as depicted by single cell gel (comet) and micronucleus assays. In the same way, apple juice concentrate was able to decrease the alkylation induced genotoxicity and oxidative DNA damage in liver cells. A decrease for 8OHdG expression in hepatocytes of animals exposed to cadmium and treated with apple juice was detected, as well. The results showed a significant decrease at catalase expression at Cd+AJ group compared to Cd group. CuZn-SOD and Mn-SOD expression did not show remarkable differences among groups.

CONCLUSION: In summary, our results suggest that apple juice exerts antioxidant and antigenotoxic activities against cadmium exposure rats.

ESTP Poster Abstracts

TP40: Experimental intoxication of guinea pigs with *Ipomoea carnea*: correlation between effects on peripheral blood and bone marrow

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Introduction: The guinea pig is an appropriate model of *I. carnea*-induced lysosomal storage disease in ruminants. This is characterized by abnormal behavior of affected animals, weakness and loss of appetite, ataxia, recumbence and death. The objective of this study was to show time-dependent intoxication, resulting in hematological and bone marrow (BM) changes; and to determine a correlation between them.

Materials and Methods: Guinea pigs (n = 12) were randomly divided into three groups of 4 animals, named a control group and two groups treated during 20 and 40 days. Pellets containing 30% of *I. carnea* powered leaves were administered to each treated animal. On the day of euthanasia, the whole blood was collected for hematological examination. Bone marrow (BM) was collected from the cavity of the femur, centrifuged, the cell´s pellet was suspended in PBS and the cells were counted in a hemocytometer. Besides, cytological examinations of BM smears were performed for cellular changes.

Results: All treated guinea pigs had significant reduction in hemoglobin concentration, PCV and RBC after 20 days of treatment compared to the guinea pigs in the control group. Additionally, globular osmotic resistance (GOR) was not changed. BM aspirates revealed decreased overall cellularity in animals receiving *I. carnea* for up to 20 and 40 days when compared to the controls. The histology of bone marrow smear from both treated groups, mainly revealed presence of less erythroid series, compared to the control group. In addition, the Myeloid-Erythroid (M:E) ratio was 0.88±0,29; 2.38±0,10 in animals treated for 20 and 40 days, respectively. The ratio 1.33± 0.03 was observed for the control group.

Conclusions: All treated guinea pigs showed haematological alterations. These animals developed normocitic and normocromic anaemia, which correlated with the BM cellularity effects. The increase in the M:E ratio at 40 days of intoxication was due to erythroid hypoplasia, associated with anaemia of medullar origin.

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ESTP Poster Abstracts

TP41: Uterine changes in a guinea pigs model of induced α – mannosidosis following *Astragalus pehuenches* intoxication

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Introduction: The ingestion of plants containing swainsonine (a potent inhibitor of alpha-mannosidase enzyme) causes intoxication characterized by nervous signs, emaciation and reproductive failure in livestock. Previous reports demonstrated reproductive problems such as cessation of spermatogenesis in adults, skeletal deformities in newborns and abortions. However, there are not studies focused on the uterus. In this work we analyzed the uterus from guinea pigs experimentally intoxicated by *Astragalus pehuenches*.

Material and methods: A group of six adult non-pregnant female guinea pigs was fed during fifty-four days with pellets containing 33% of *A. pehuenches* (IG). The control group (CG) received similar pellets without the toxic plant. The animals were sacrificed and perfused with 4% paraformaldehyde. Uterine samples were processed for conventional histological study, transmission electronic microscopic (TEM), and carbohydrate analysis by histochemistry (PAS, Alcian blue, lectinhistochemistry).

Results: Vacuolation of cytoplasm in uterine luminal and glandular epithelium and myometrium cells was observed. The stain of the uterine secretions in IG guinea pigs was lower than in CG as assessed by conventional histological study. In the TEM study, the vacuoles had a single membrane and contained electron-dense membranous structures/fragments. The histochemical study demonstrated a change in the intensity and/or histological location of the carbohydrates principally by biding LCA (α-Glc, α-D-Man), PNA (Gal-α (1-3)-GalNAc), DBA (α-linked N-acetyl galactosamine) and PHA-L (bisected triantennary N-glycan).

Conclusions: Vacuolation in the cytoplasm of uterine luminal and glandular epithelium and myometrial myocytes seems to be the result of lysosomal carbohydrate storage as assessed by lectin histochemistry study. In previous reports, similar lectin pattern biding on nervous tissue of spontaneous and experimental intoxicated animals with alpha-mannosidosis was observed. The guinea pig model could be used to evaluate the reproductive disorders observed in intoxicated livestock.

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ESTP Poster Abstracts

TP56: Protective effects of intranasal immunization with recombinant glycoprotein in the BALB/c mouse model of Equine herpesvirus 1 infection

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Introduction: Equine Herpesvirus 1 (EHV-1) causes abortion, respiratory and neurological disorders in horses. Natural respiratory infection and immunoprophilaxis do no provide long-lived protection. We evaluated the protection conferred by a purified baculovirus-expressed glycoprotein D (gD) to BALB/c mice challenged with the Argentinean AR8 EHV-1 strain.

Material and Methods: Thirteen groups of BALB-c mice were immunized with gD (two doses of 15 µg each) or phosphate buffered saline (control groups) using parental or intranasal (IN) routes and Complete Freund, Specol or Montanide, as adjuvants. Immunoglobulin G (IgG) detection and virus neutralization was determined in pool blood samples. Mice were challenged with ~106.3 TCID50/ 50 µl of EHV-1 AR8 by IN route. Infectivity titration, histopathological and immunohistochemical studies were done in lungs.

Results: Mice immunized by parenteral routes showed clinical signs, positive virus isolation, lesion in lungs, and an effective IgG response. After IN immunization no clinical sign was observed, virus isolation was negative, no lesion or viral antigens were detected. The IgG response was not efficient, but IgA was detected in bronchiolar epithelium.

Conclusions: Our results support the IN immunization to prevent the virus entry to the lungs and provide novel information with respect to immunization routes. Support. This will lead to propose new strategies to test gD immunization on natural hosts at critical moment for the natural infection.

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ESTP Poster Abstracts

TP42: Genotoxicity and cytotoxicity induced by municipal effluent in Wistar rats

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The aim of this study was to evaluate cytotoxicity and genotoxicity induced by municipal effluent in rats. A total of 21 male Wistar rats (8 weeks old from Federal University of Sao Paulo) were exposed to drinking water from effluents *ad libitum* at concentrations of 0, 50 and 100% for 30 days (seven animals per group). Microscopic analysis revealed severe lesions such as necrosis, hemorrhagic areas and the presence of inflammatory infiltrate in the liver from animals exposed to effluent at 50% and 100% concentration. DNA damage in peripheral blood, liver and kidney cells were detected by comet assay at higher concentrations of effluent (50% and 100%). Moreover, a decreased DNA repair capacity was detected in liver cells. Significant statistically differences ($p<0.05$) for micronucleated cells from liver were noticed at 50% concentration of effluent. Taken together, our results demonstrate that municipal effluent is able to induce cytotoxicity and genotoxicity in multiple organs of Wistar rats.

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TP43: Mimosa (*Mimosa caesalpiniifolia)* modulates chemically-induced genotoxicity by cadmium exposure in liver and blood cells of Wistar rats

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The Mimosa (*Mimosa caesalpiniifolia*) is a plant native from South America; it is used in the traditional medicine systems for treating bacterial, fungal, parasitic and inflammatory conditions. The aim of this study was to evaluate the antigenotoxic and antioxidant properties of mimosa in Wistar rats subjected to intoxication with cadmium chloride.

A total of 35 male Wistar rats (8 weeks old, 250g) were distributed into seven groups (n=5), as follows: Control group (non-treated group, CTRL); Cadmium exposed group (Cd); cadmium exposure and treatment with mimosa extract at 62.5 mg/kg/day; cadmium exposure and treatment with mimosa extract at 125 mg/kg/day; cadmium exposure and treatment with mimosa extract at 250 mg/kg/day; cadmium exposure and treatment with ethyl acetate fraction at 62.5 mg/kg/day. In order to evaluate the toxicogenetic potential of mimosa extract, two additional groups were included in this study and treated with extract at 250 mg/kg/day and acetate fraction of mimosa at 62.5 mg/kg/day, respectively.

Extract of mimosa at concentrations of 62.5 and 125 mg decreased DNA damage in liver cells from animals intoxicated with cadmium when compared to cadmium group. In a similar manner, treatment with ethyl acetate fraction of mimosa at 62.5 mg concentration reduced genetic damage in peripheral blood and liver cells. Oxidative DNA damage was reduced at 125 mg of mimosa extract as well as 62.5 mg of acetate fraction of mimosa.

Taken together, our results indicate that mimosa prevents genotoxicity induced by cadmium exposure in liver and peripheral blood cells of rats as a result of antioxidant activity.

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TP45: Exogenous human recombinant thioredoxin is preventive against acetoaminophen-induced hepatotoxicity by reducing peroxynitrite production and inhibiting degradation of endogenous thioredoxin

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Thioredoxin-1 (Trx-1) has multiple functions including anti-oxidation, anti-inflammation and anti-apoptosis, but excessive oxidative stress induce degradation of Trx-1, resulting in cell injury. Overdose of acetaminophen (APAP) causes hepatotoxicity by generation of reactive nitrogen species, mitochondrial dysfunction, and DNA damage. With this background, we investigated if exogenous treatment of human recombinant thioredoxin protein (rhTrx) can prevent the acetoaminophen-induced hepatotoxicity and explored the underlying mechanism. For the study, C3H mice were pretreated with human recombinant Trx-1(rhTrx-1 at 10, 50 or 100 mg/kg), followed by administration of 300 mg/kg b.w. APAP, and then sacrificed after 6h. To see the potential effect of rhTrx-1 on survival, the mice were treated with rhTrx-1(10 mg/kg) or saline 30 min before oral administration of a lethal dose of APAP (400 mg/kg b.w.) and then monitored up to 72h. After gross examination, formalin-fixed livers were prepared for histological examination. The necrotic areas in the livers were quantitated by image analysis. TUNEL assay and immunohistochemistry for 3-nitrotyrosine were carried out to detect the apoptotic and peroxynitrite-affected cells, respectively. Western blotting was also performed to quantitate peroxynitrite and Trx-1 level in the liver. As a result, pretreatment of rhTrx-1 markedly reduced the APAP-induced hepatocyte necrosis and apoptosis, and exogenous rhTrx-1 decreased the lethality of APAP up to 50%, compared to the vehicle group. These results are likely related to the inhibitory effect of rhTrx-1 against the nitrotyrosine production following APAP treatment. Exogenous rhTrx-1 also maintained the effective level of Trx-1 in the liver that was markedly decreased by APAP. According to these results, we could conclude that rhTrx-1 ameliorates APAP-induced liver injury through reducing peroxynitrite production and inhibiting degradation of endogenous Trx-1.

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TP46: Acute oral, subchronic oral and dermal toxicity studies in Sprague Dawley rats treated with the agrochemical insecticide Accusave 50%FS (FIPRONIL 50%)

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Introduction: Acute oral, subchronic oral and dermal toxicity studies of Accusave-50%fs (Fipronil 50%), an agrochemical insecticide, in adult Sprague-Dawley rats, were conducted for safety use and applications.

Materials and Methods: Eight rats were used in each of the treatments and the untreated control group. Initial and final body weights (BW) of rats and organ weights (OW) were recorded. Tissues and blood samples were also collected and examined.

Results: Analysis of the results showed that the acute oral toxicity (LD50 in 48 hours) is greater than 100mg/kg. The subchronic oral toxicity studies (14 days with 10 mg/kg per day) revealed no significant body weight gain in the treated group compared to the control group; 1% in the treated vs 14% in the control. The ratio of the means of the liver and kidney to the mean BW in the treated group was decreased but not significantly compared to the control. There was a slight increase in the spleen weight in the treated group compared to the control. Packed Cell Volume (PCV) and Hemoglobin (Hgb) percentage were decreased in the treated group compared to the control. The total plasma proteins, ALT and AST were increased in the treated group compared to the control. In addition, the results of the subchronic dermal toxicity study (14 days with 17 mg/kg, dermal application-once daily) indicated similar changes; there was a slight increase in the mean of the body weight in the treated compared to the control group (4% vs 17%) when compared with the mean of starting weights. The ratio of the mean weight of the liver, however, was increased almost two fold in the treated group compared with the control (0.042% vs 0.028%).There was no significant change in RBC, PCV, Hgb and WBC while an minor (non significant) increase in total proteins, ALT and AST in the treated group compared to the control group was observed. There were multifocal foci of necrosis in the liver and kidneys, with increased Kupffer cells in the liver sinusoids in the orally treated group. The focal necrosis in the kidneys was limited to the proximal tubules. These changes were not seen in dermal toxicity group. In addition, no significant histopathological alterations were seen in the skin or any other organs examined as a result of the 14 days dermal or oral administration.

Discussion (and/or Conclusions): These three studies collectively suggested that Accusave-50%fs (Fipronil 50%) is moderately toxic, and is classified in category 3 in the Global Harmonized System (GHS)

ESTP Poster Abstracts

TP47: Non-clinical safety assessment of Guibi-Tang: Subchronic toxicity study in Crl:CD SD rats

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Guibi-tang (Qui-Pi-Tang in Chinese and Kihi-To in Japanese) is a multi-herbal traditional Korean medicinal formula used for treatment of treat amnesia, poor memory or forgetfulness, fatigue, insomnia, anemia, palpitation, and necrosis in man. The objective of the present study was to investigate potential adverse effects, if any, of subchronic administration of Guibi-tang aqueous extract (GBT) in male and female rats. For this study, 0, 1000, 2000, and 5000 mg/kg/day of GBT was administered to Crl:CD Sprague Dawley rats (10/sex/group) for 13 weeks via oral gavage. Administration of the GBT did not result in any mortality. There were no clinical or ophthalmological signs, changes in urinalysis, body weight, food consumption, gross findings, hematology, serum biochemistry, organ weight or histopathology attributable to administration of GBT. Any changes noted were incidental and consistent with those historically observed in the age and strain of rats used in this study. Based on the results of this study, the no-observed-adverse-effect level for GBT under the present experimental conditions was determined to be 5000 mg/kg/day, the highest dose tested, for both sexes.

Key words: Guibi-tang, Qui-Pi-Tang, Kihi-To, subchronic, NOAEL

ESTP Poster Abstracts

TP48: Effect of curcumin on differentiation and migration of human circulating fibrocytes in vitro.

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Circulating fibrocytes were discovered by Bucala in 1994, and recent studies have confirmed that they may play important roles in the formation and development of fibrosis. Curcumin, a polyphenolic compound isolated from turmeric (Zingiberaceae), has been shown to exhibit anti-fibrosis activity in various organs[1-4]. Here, we explored curcumin's ability to modulate the proliferation, activation, differentiation and migration of human circulating fibrocytes. Human circulating fibrocytes were isolated from the leukocyte concentrate of healthy human donors. Following isolation and purification by density gradient centrifugation, these cells were shown to be positive for COL1, CD45 and CCR7 identified by flow cytometry and immunocytochemistry.

cells were treated with curcumin (0, 1, 3, 10, 20μmol/L; 72h), The effect of curcumin on fibrocytes activation was observed by flow cytometry ,while Cell viability and migration were studied by Cell Counting Kit-8 and Transwell migration assay respectively. ELISA was used for the expression of TGF-β1.

Curcumin treatment at high dose and prolonged time (72h; 20umol•L^{-1}.) significantly reduced cells propagation, downregulated the COL I, α-SMA and CCR7 levels, as well as the TGF-βl secretion. CCR7/CCL21 signal pathway could induce cells recruitment and curcumin could hold the migration of human circulating fibrocytes via blocking the signal pathway, chiefly via reducing CCR7 expression. These effects of curcumin on human circulating fibrocytes may contribute to resolving the fibrosis, which is the first report to describe the effect of curcumin on human circulating fibrocytes in vitro.

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ESTP Poster Abstracts

TP49: Relationship of coumarin-induced hepatocellular toxicity and subcellular distribution of cytochromes P450 in the rat

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Background: Several cytochromes P450 (CYPs) are located not only in the endoplasmic reticulum but also in the mitochondria. Such CYPs include CYP1A and 2E1 which mediate the production of reactive metabolites, and it is not clear if the mitochondrial targeting CYPs play a role in drug-induced liver injury. Coumarin, which is an aromatic compound, is known as a flavor component of cinnamon. In the rat, 3, 4-epoxide and o-hydroxyphenylacetaldehyde, which are reactive metabolites of coumarin metabolized by CYP1A and 2E1, induce severe hepatotoxicity. Therefore, in this study, we examined the influence of coumarin on subcellular distribution of CYP1A1 and 2E1 and mitochondrial function in the rat.

Methods: Coumarin (300mg/kg) or vehicle (corn oil) was administered orally 1 or 4 times, and the animals were sacrificed 4 or 24 hours after single dosing and 24 hours after 4-time dosing. Measurement of the liver enzymes and histopathological examination were performed. Furthermore, immunohistochemical examination of prohibitin, a mitochondrial marker and subcellular distribution of CYP1A1 and 2E1 were carried out.

Results: In coumarin treated group, at 4 hours after a single dosing, eosinophilic granular change of the centrilobular hepatocytes was observed. At 24 hours after a single dosing, severe centrilobular necrosis with infiltration of inflammatory cells was seen. On the other hand, at 4-time dosing, necrotic change was not detected, and centrilobular hepatocytes had eosinophilic granular cytoplasm. Thus, necrotic hepatotoxicity was improved by repeated administration of coumarin. Immunohistochemically, prohibitin was strongly positive in the granules of centrilobular hepatocytes which observed at 4 hours after single dosing and 4-time dosing. CYP1A1 and 2E1 were diffusely distributed in cytoplasmic granules of centrilobular hepatocytes in the control group. And in this group, double-positive granules with prohibitin and CYP1A1 /2E1 were also observed. At 4 and 24 hours after single dosing, there were no clear changes of the subcellular distribution of CYP1A1 and 2E1 compared with control group. On the other hand, at the 4-time dosing, the distribution of CYP2E1 was change diffuse to multifocal. And the form of CYP2E1 positive granules were also change micro granular to droplet. Additionally, the double positive granules with prohibitin and CYP2E1 were decreased at the 4-time dosing. The subcellular distribution of CYP1A1 did not change compared with control group and single dosing.

Conclusion: These findings suggested that mitochondrial targeting CYP2E1 played an important role in hepatocellular toxicity and acquisition of the resistant for coumarin toxicity.

ESTP Poster Abstracts

TP50: Light and transmission electron microscopical changes associated with *leiurus quinqestriatus* venom in rabbits

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Thirty California female rabbits were obtained from the Animal Care Center, College of Agriculture, South Valley University and acclimated to laboratory conditions for one week. The Leiurus quinquestriatus (LQ) venom was collected from mature scorpions by electrical stimulation of the telson. A single dose of crude venom of 0.4 ml/kg (diluted in normal saline with a ratio of 1:1) was injected into a peripheral ear vein. The lungs, brains, hearts, kidneys, spleen, and ovaries were sampled and fixed in 10% formalin from rabbits sacrificed at 0, 30 minutes, 1hr, 2hr, 4hr, 8hr, one day, 2 day and 8 day post-envenomation (three animals at each sacrifice).

Respiratory distress and neurological manifestations were the main clinical signs. Congestion of the lungs started at one hour post-envenomation. Vascular changes including hyperemia and haemorrhage were also observed till 24 hours post-envenomation. Edematous appearance of the lungs was observed mainly at one day and returned normal by 8 day post envenomation. The main histopathological changes of the lungs were edema, haemorrhage, emphysema, and eosinophilic bronchitis. Transmission electron microscopy revealed several eosinophils with abundant granules and breakdown of their membranes suggesting degranulation.

The cerebrum showed malacia and edema. Myocardial damage expressed by focal area of myolysis at half-hour post-envenomation and interstitial edema by at 1, 2, and 4 hour post- envenomation was also evident. Nephrotoxicity was observed up to 24 hours post- envenomation, while oophoritis was mainly observed at 24 hours envenomation.

These results were correlated with the hematological and biochemical parameters such as, RBCs count HB, PCV, MCV, MCH, MCHC. ALT and AST, total Plasma protein, Glucose assay, Creatinin, Urea and Cholestrol. RBCs count showed significant decrease as early as 2h post exposure until day 4, while become similar to control after 8 days from envenomation. Both Hb and PCV values were similar to RBCs values. The values of MCV, MCH, and MCHC showed non-significant changes at most of the tested time points. The results of both hepatic cell necrotic indicators; AST and ALT; showed significant increase at day 1 after exposure compared to the control. While, the values of total plasma proteins showed significant decrease as early as 1 hour post exposure and reach to the minimum values at day 1 after exposure. Glucose assay revealed non-significant changes at all of the tested time points except at day 1 after exposure where there is significant decrease. Both urea and creatinine showed non-significant changes at all of the tested time points. Cholesterol values revealed non-significant decrease at most of the tested time points.

In conclusion, scorpion venom induced consistent and relevant histopathological changes in all examined organs as indicated by significant decrease of RBCs as early as 2h post exposure until day 4, while become similar to control after 8 days from envenomation. While, the values of total plasma proteins showed significant decrease as early as 1h post exposure and reach to the minimum values at day 1 after exposure.

ESTP Poster Abstracts

TP51: Evaluation of xenotransplanted human adipose stem cells efficacy in a mouse model of skin burn wound healing

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Introduction: Cutaneous wound healing in man is a major health concern particularly after burn. The ultimate goal is to be able to regenerate skin with structural and functional properties of uninjured skin. Human Adipose Derived Mesenchymal Stem/Stromal cells (hASC) may support tissue recovery through angiogenesis, paracrine effects and modulation of the inflammatory response. Local hASC delivery thus represents a promising strategy in regenerative medicine for cutaneous wound healing.

Aim: To evaluate the influence of transplanted hASC on wound healing in a mouse model of cutaneous burn injury.

Materials and Methods: hASC, obtained as previously described[1], or vehicle (0.9% NaCl) were subcutaneously injected into the burned wound bed of 25 nude mice. The site was evaluated up to 21 days postoperatively. Formalin-fixed, paraffin-embedded samples were evaluated for wound healing criteria.

Results: Gross evaluation follow-up focused on wound surface measurement, determination of skin contraction index and time for complete re-epithelialization. hASC enhanced wound closure and reduced scar formation. Laser Doppler imaging was improved from D7 to D21 by hASC delivery.

Histological evaluation included variations over time in re-epithelialization, immune/inflammatory infiltrates, neovascularization, regeneration of skin appendages and scar thickness. The differences between hASC and vehicle-control wounds are discussed in the light of hASC biological properties. Immunohistochemistry showed the hASC survived transplantation and integrated into the scarring tissue, in particular in perivascular location.

Conclusion: These preliminary results support the feasibility and promising potential of hASC delivery for the treatment of cutaneous burn injuries, by improving the wound healing process and the biological properties of the scar in nude mice.

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ESTP Poster Abstracts

TP52: Metal exposure and toxicology in selected fish species from the Mediterranean sea: risk assessment for human consumption

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Introduction: Myriads of toxic substances are released into our environment daily, either deliberately manufactured or accidentally produced. Aquatic systems throughout the world are increasingly under a wide array of anthropogenic stressors. However, some of aquatic environments can sustain fish populations, indicating that they are able to tolerate toxic levels of metals.

Aim: The concentration levels of different chemical compounds in pelagic and benthopelagic fish species from the Mediterranean sea were examined by testing specific tissues involved in the bioaccumulation pathway with GC/MS.

Materials and Methods: Fish samples were collected monthly from April 2013 to September 2013. No abnormalities were evidenced on macroscopical examination. The presence of twenty-seven heavy metals and minerals (Hg, Na, Mg, Al, K, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Ag, Cd, Sn, Sb, Ba, Tl, Pb, Th, U) was investigated in livers and muscles collected from *Pagellus bogaraveo*, *Dentex dentex*, and *Thunnus thynnus* from the Mediterranean sea.

The mean concentration of each element was calculated. A comparison to the provisional tolerable daily or weekly intake or to the tolerable upper intake established by the Joint FAO/WHO Expert Committee on Food Additives and the EFSA was assessed. The maximum safe consumption (MSC) for adult intakes was achieved for each element with an established safety limit.

Results: Hg, Fe, Co, Ni, Zn, As, Se, Sr, Mo, Cd, Sn, Ba were variably present at higher level within muscular samples of the 3 different fish species. The MSC calculated for mercury lead to a limited recommended weekly intake for all the tested fish species.

Conclusions: This study provides preliminary information on metal concentration in the edible part of three commercial fish species. Based on MSC, mercury concentration in muscle of *Pagellus bogaraveo*, *Dentex dentex*, *Thunnus thynnus* exhibits a risk for human consumption.

ESTP Poster Abstracts

TP53: Pathology Working Group Review of histopathologic specimens from three laboratory studies of Diclofenac in trout

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Background: A Pathology Working Group (PWG) panel evaluated histopathologic sections from three prior studies of diclofenac in trout (Hoeger et al. 2005, Mehinto et al. 2010, Memmert et al. 2013). Data of a fourth pivotal trout study (Schwaiger et al. 2004) were unfortunately not available for review.

Methodology: Following a complete examination of all histologic sections and original diagnoses by a single experienced fish pathologist (pathology peer review), a two-day PWG session was conducted to allow members of a four-person expert panel to review a subset of the histologic sections and determine the extent of treatment-related findings in each of the three trout studies. In accordance with standard procedures, the PWG review was conducted by the non-voting chairperson in a manner intended to minimize bias. The four voting panelists were unaware of the treatment group status of individual fish and the original diagnoses associated with the histologic sections.

Results: Based on the results of this PWG review, findings related to diclofenac exposure included minimal to slightly increased thickening of the gill filament tips in fish exposed to the highest concentration tested (1000 µg/L), plus a previously undiagnosed finding, decreased hepatic glycogen, which also occurred at the 1000 µg/L dose level. The panel found little evidence to support other reported effects of diclofenac in trout, and thus the overall NOEC was determined to be 320 µg/L. On the other hand, the panel noted diagnostic inconsistencies within and among the previous studies, including a lack of diagnoses in the control group in one study, and technical shortcomings in another case.

Conclusions: This exercise clearly demonstrated the importance and added value of using the pathology peer review/ PWG approach to assess the reliability of histopathology results that may be used by regulatory agencies for risk assessment purposes. The analytical power of a PWG is far greater than that of a journal peer review in which the actual histologic slides are not available for examination. For the reliable derivation of Environmental Quality Standard (EQS) values, a PWG approach is highly recommended.

ESTP Poster Abstracts

TP54: Non-lesions, misdiagnoses, missed diagnoses, and other interpretive challenges in fish histopathology studies: A guide for investigators, authors, reviewers, and readers

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Introduction: Differentiating salient histopathologic changes from normal anatomic features or tissue artifacts can be especially challenging for the novice fish pathologist. Consequently, findings of questionable accuracy may be reported inadvertently. The objectives of this project were to identify specific morphologic findings in commonly examined fish tissues that are frequently either misdiagnosed or underdiagnosed, and to illustrate such findings through the use of photomicrographic examples.

Experimental Design and Methods: A number of highly-trained, veteran fish pathologists were tasked with assembling lists of histopathologic diagnoses that often appeared questionable based on evaluations of published morphologic descriptions and figure illustrations. For the current project, photomicrographic examples of normal and abnormal specimens were acquired from the personal slide collections of the authors, or obtained by permission from prior studies.

Results: Histopathologic findings that appeared to be commonly over-diagnosed or misdiagnosed in the literature included nine types of gill diagnoses, six kidney diagnoses, four liver diagnoses, and five additional diagnoses in various other tissues. Additionally, the authors identified nine types of findings that tend to be under-reported.

Conclusion: Histopathology continues to be a valuable tool for investigating the morphologic features and extent of both naturally-occurring and experimentally-induced disease. The authors describe practical measures that can be instituted to safeguard against the publication of dubious histopathologic results.

Impact statement: The fundamental goal of this effort is to elevate the science and practice of fish histopathology, which has become an increasingly important discipline in fields that include basic biomedical research, aquaculture, environmental resource management, and ecotoxicology.

ESTP Poster Abstracts

TP55: Genetically obese mice are resistant to lethal effects of thioacetamide hepatotoxicity

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Obesity increases the risk of chronic liver diseases, including viral hepatitis, alcohol-induced liver disease and non-alcoholic steatohepatitis (NASH). In this study, we investigated the involvement of obesity in acute hepatic failure (AHF) using a thioacetamide (TAA)-induced liver injury model. The ob/ob mice and their littermates, ob/+ were subjected to a single intraperitoneal TAA injection, and various parameters of hepatic injury were assessed. The ob/ob mice displayed a significantly higher survival rate, lower serum alanine aminotransferase and aspartate aminotransferase levels, and less hepatic damage, compared to ob/+ mice. In addition, the level of oxidative stress was significantly less in ob/ob mice than in their ob/+ counterparts, as evident from lipid peroxidation assay. Importantly, analysis of TAA bioactivation revealed lower plasma clearance of TAA and covalent binding of [14C]TAA to liver macromolecules in ob/ob mice. These results collectively indicate that obesity in mice protects against TAA-induced acute liver injury via lower bioactivation of TAA and antioxidant effects.

ESTP Poster Abstracts

TP57: Comparative study of central nervous lesions in sheep intoxicated by *Astragalus pehuenches*

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Introduction: The first outbreak of *Astragalus pehuenches* poisoning in sheep was reported in Argentina in 2000. By 2012, high concentrations of swainsonine (SW) were detected in *A. pehuenches*. The pattern of lesions of the central nervous system (CNS) derived from experimental and natural poisoning of sheep by *A. pehuenches* was compared.

Materials and Methods: Four Merino ewes were used in this study. Ewe1 and Ewe2 were experimentally intoxicated with *Astragalus pehuenches* to obtain SW dosages of 1 and 2mg/kg/day during 54 days, respectively. Ewe3 was naturally intoxicated during the outbreak of 2000. Ewe4, never exposed to *A. pehuenches*, was used as control. Formalin-fixed sections of CNS tissue were routinely processed, stained with HE and examined under a light microscope.

Results: Cytoplasmic vacuolation was the most remarkable finding in Ewe1, Ewe2 and Ewe3. The pattern of lesion varied from distended neurons with foamy cytoplasm containing numerous small vacuoles and dispersed Nissl substance up to neurons showing total disruption of cytoplasm. Purkinje cells, neurons of deep cerebellar nuclei, hypoglossal nuclei and medullary reticular lateral neurons were the most affected. The histopathological picture observed in Ewe2 resembled that in the naturally intoxicated sheep (Ewe3), being lesions in Ewe 1 less severe.

Conclusions: Based on this histopathological study, we can conclude that experimental intoxication in sheep by *Astragalus pehuenches* was possible and suggest a link between this poisoning and acquired α-mannosidosis. Our results determined that the lesions in sheep intoxicated with 2mgSW/kgBW resemble, in severity, the ones of naturally intoxicated sheep. These results should allow us to characterize clinically, biochemically and histopathologically the poisoning by *A. pehuenches*.

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THE EUROPEAN SOCIETY OF VETERINARY PATHOLOGISTS 33RD ANNUAL MEETING & THE 26TH ANNUAL MEETING OF EUROPEAN COLLEGE OF VETERINARY PATHOLOGISTS

will take place jointly with the meeting of Nordic Society of Veterinary Pathology on

the 2nd to 5th of September 2015, in Helsinki, Finland

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VENUE

The meeting will be held in Helsinki at the Marina Congress Center in Katajanokka. Marina Congress Center is a congress venue with the latest meeting technology located at the waterfront in the center of the city.

PROGRAM

Wednesday Sept 2nd, Registration, Get together and University of Helsinki Reception
Thursday Sept 3rd, Full day conference, City of Helsinki Reception
Friday Sept 4th, Full day conference, Congress dinner
Saturday Sept 5th, ½ day ESVP-ECVP conference, ½ day NSVP annual meeting with mystery slide sessions

PRE-CONFERENCE MEETING ON THE 1ST & 2ND OF SEPTEMBER: a course for anatomic pathologists on the cytology of hematopoietic disorders and tumor masses. Lecturers: Prof. Mary Anna Thrall USA and Prof. Donald Meuten USA. The course is organized by Nordic Society of Pathology/Evira chapter. (Inquiries: Veera Karkamo veera.karkamo@evira.fi)

POST-CONFERENCE MEETING: one and half day C. L Davis symposium 5th and 6th of September.
(Inquiries: Liz McInnes efmcinnes@aol.com)

We are looking forward to seeing you in Helsinki and welcome you to join us for the ESVP-ECVP 2015 Meeting on behalf of the ESVP and ECVP Local Organizer Committee:

Prof Antti Sukura, University of Helsinki
Prof Marjukka Anttila, Finnish Food Safety Authority Evira
Clinical teacher Pernilla Syrjä, University of Helsinki

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