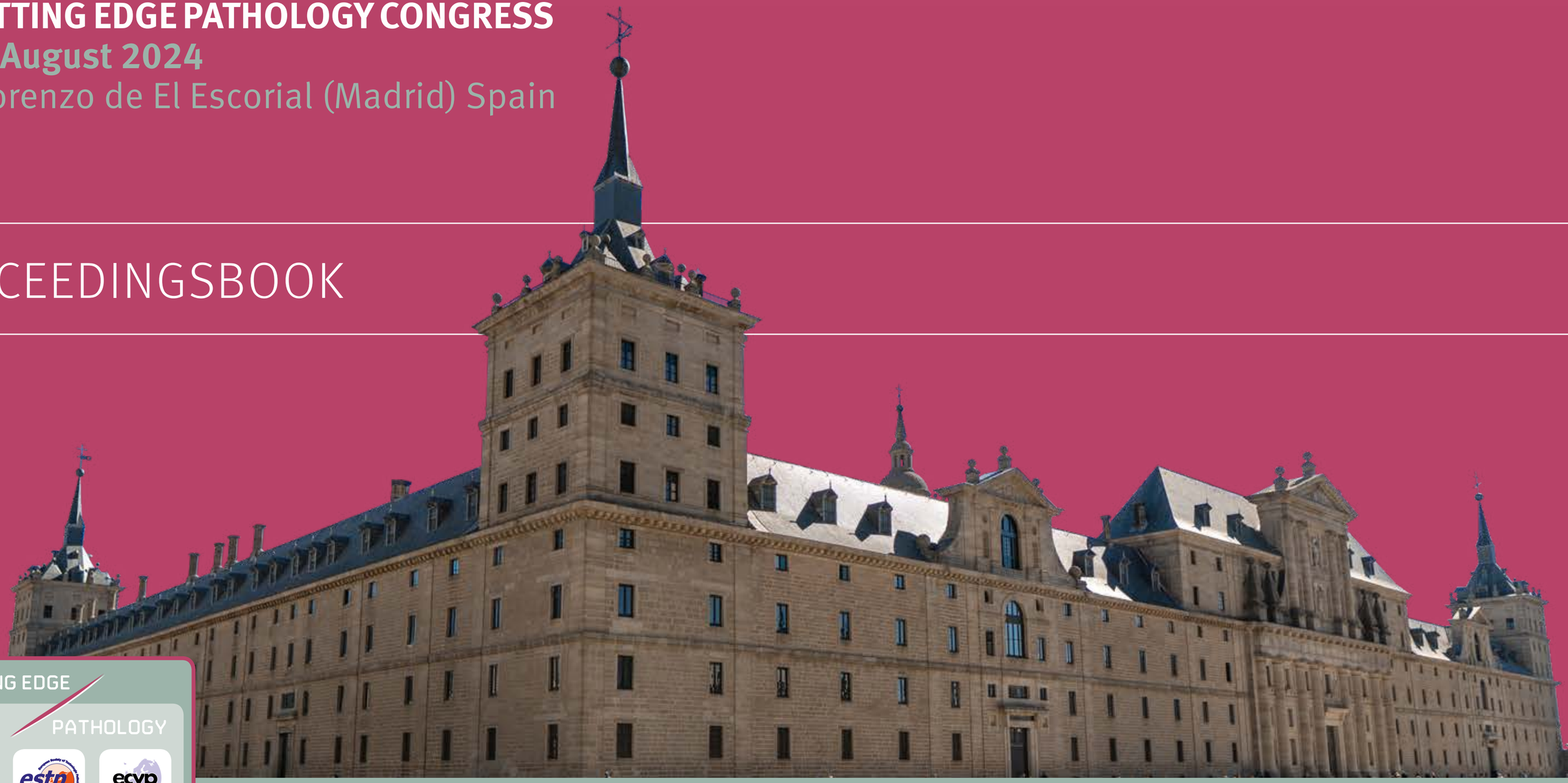
5TH CUTTING EDGE PATHOLOGY CONGRESS

28-31 August 2024

San Lorenzo de El Escorial (Madrid) Spain

PROCEEDINGSBOOK



CUTTING EDGE PATHOLOGY

WELCOME TO SAN LORENZO DE EL ESCORIAL

28-31 AUGUST 2024

About four years ago, we considered the possibility of having this wonderful congress in Madrid. It seemed very far away. The Local Organizing Committee (LOC) began to look for locations and finally, we found this magnificent and emblematic site in San Lorenzo de El Escorial.

For the preparation of this congress, there have been several years of work filled with enthusiasm and, it must be said, concerns and moments of discouragement. In this task, we have always tried to offer you the best in order to provide you with a cutting-edge congress that is as pleasant as possible in its organizational aspects and at the same time scientifically interesting (thanks Scientific Committees!).

It has been a great satisfaction to see the high number of registered congress participants and we hope not to disappoint you in the slightest detail.

We do not want to miss mentioning that this congress will also be special for us within the field of Veterinary Pathology in Spain, as we expect to have numerous members of the Spanish Society of Veterinary Anatomical Pathology (SEAP) who will celebrate their thirty-fifth annual meeting here in Madrid... and we are very demanding with our national SEAP congresses since the bar is set very high!

With certain nervousness while waiting for everything to go well, now we just need to enjoy Science, Veterinary Pathology, and friends!

We hope to see you soon!

Laura Peña
Chair of the LOC

Juana M Flores
Co-Chair of the LOC

THE EUROPEAN SOCIETY OF VETERINARY PATHOLOGY

Dear ESVP and ESTP Members, dear ECVP Diplomates, dear Colleagues, dear Friends,

On behalf of the Board of the European Society of Veterinary Pathology, this is my great pleasure to welcome you to the 5th Cutting Edge Pathology (CEP) Congress, in the wonderful city of San Lorenzo del Escurial, Spain.

The European Society and College will indeed co-organize this year our annual Congress with our friendly and sister partner, the European Society of Toxicological Pathology (ESTP) at the Real Centro Universitario Maria Cristina in front of the impressive building of the Real Monasterio of San Lorenzo del Escurial. As every 3 years, this Cutting Edge congress is special as the conference will be a joint venture between the European Society of Veterinary Pathology, the European Society of Toxicologic Pathology, and the European College of Veterinary Pathologists with an extensive program covering veterinary pathology as well as toxicologic pathology. The meeting will enable both disciplines of pathology to learn from each other's work and we hope it provides opportunities for new collaborative ventures between veterinary and toxicologic pathologists. It will be in addition a wonderful opportunity to strengthen the natural link between our communities. After the great sucesss of the Lisbon meeting last year, we had another proof, if needed, how such events are crucial in terms of sociability and human interactions among our community. The increased number of young colleagues, residents and PhD Students attending ours Meetings, are also a promising feature and a strong encouraging signal. Working for them is our honour. This 5th CEP in 2024 will be a wonderful opportunity to strengthen the natural link between our communities, of young residents and experienced pathologists, either working in diagnostic pathology, in academias, or in toxicological Pathology. The Local Organizing Committee team, head by Laura Peña, with the help of our PCO (special thank to Mirel Kostons), has remarkably organized this event. And the program that has been built by both Scientific Committees of the ESVP-ECVP and of the ESTP will undoubtedly cover our expectations.

I am endly sure that you will all enjoy the beautiful city of San Lorenzo del Escurial and the spanish hospitality and way of life. Looking forward to see you all soon.

Sincerely and kindly. Best regards,

Jérôme Abadie
President ESVP

THE EUROPEAN COLLEGE OF VETERINARY PATHOLOGY

Welcome everyone! Welcome to our 5th cutting edge pathology congress. This meeting, jointly organized by the European College of Veterinary Pathologists, the European Society of Veterinary Pathology and the European Society of Toxicologic Pathology, is an incredibly rich and exciting event. Our organizing teams and partners have been working hard on preparing this congress which now becomes reality. Thank you all for your relentless commitment.

First, our scientific program has been designed with outmost care to feed our professional curiosities. Whether you are specialized in research, diagnostic or toxicologic pathology, our joint congress is a unique forum to exchange about our different yet complementary practical experiences. Neuropathology, oncology, infectious and degenerative disease in the broadest range of animal species on planet Earth will trigger passionate discussions. Diverse points of view will constructively feed into our common enthusiasm for understanding and analyzing mechanisms of diseases. Innovative ideas will emerge, which each of us can take back home and transform into concrete changes in our daily works. You will also meet hundreds of talented peer pathologists. To facilitate networking, our organizers have designed a fantastic social program. Make most of this privileged moment together. Entertain across generations, across countries, across cultures. The diversity of our community is its strongest value.

Lastly, let me mention the royal site of El Escorial which is a truly unique meeting location in Europe. Close to Madrid, founded in the XVIth century by king Philippe II, it gathers a monastery, a museum, a college, a library and a palace making it a highly inspirational venue. Numerous sight-seeing opportunities should not be missed and we will all appreciate its features, echoing from the past, whilst looking ahead at our future.

Enjoy our congress, enjoy being together.

Yours sincerely.

Alexandre Bidaut
President of the European College of Veterinary Pathologists

ecvp
European College of Veterinary Pathologists

THE EUROPEAN SOCIETY OF TOXICOLOGIC PATHOLOGY

A very warm welcome to the 5th Cutting Edge Pathology congress, set against the historic and picturesque backdrop of San Lorenzo de El Escorial! I am delighted to have you with us for this important event dedicated to advancing the field of pathology, made possible by the collaboration of ESTP, ESVP and ECVP.

In the coming days, we will participate in engaging discussions, share groundbreaking research, and build collaborations that will shape the future of our field. This congress unites experts, researchers, and practitioners from across the globe and various pathology specialties, each bringing unique perspectives and innovations. This year, ESTP has chosen neuropathology as our scientific focus, a field that is also vital for diagnostic pathology. With advancements ranging from Alzheimer's treatment to the direct application of gene therapy and the implantation of microchips in the central nervous system, therapies for neurological diseases are making significant strides. Interdisciplinary thinking and awareness are essential to address the challenges these advancements present.

San Lorenzo de El Escorial, nestled in the serene Sierra de Guadarrama and steeped in cultural heritage, offers an ideal setting for our congress. I urge you to take the time to explore its historic landmarks and appreciate the natural beauty around us. We can anticipate a delightful evening at Finca Miravalle, where we will experience warm Spanish hospitality. At this point I would like to extend a big Than You! To our scientific organizing committee, led by Pierre Maliver and Sameh Youssef, for putting together a series of exciting, truly cutting- edge talks; a heartfelt thanks to Laura Peña and the local organizing committee; and special thanks to all our generous sponsors, who supported us in putting this exceptional congress together.

With my very best wishes for an enjoyable and insightful congress!

Silvia Guionaud
President, European Society of Toxicologic Pathology

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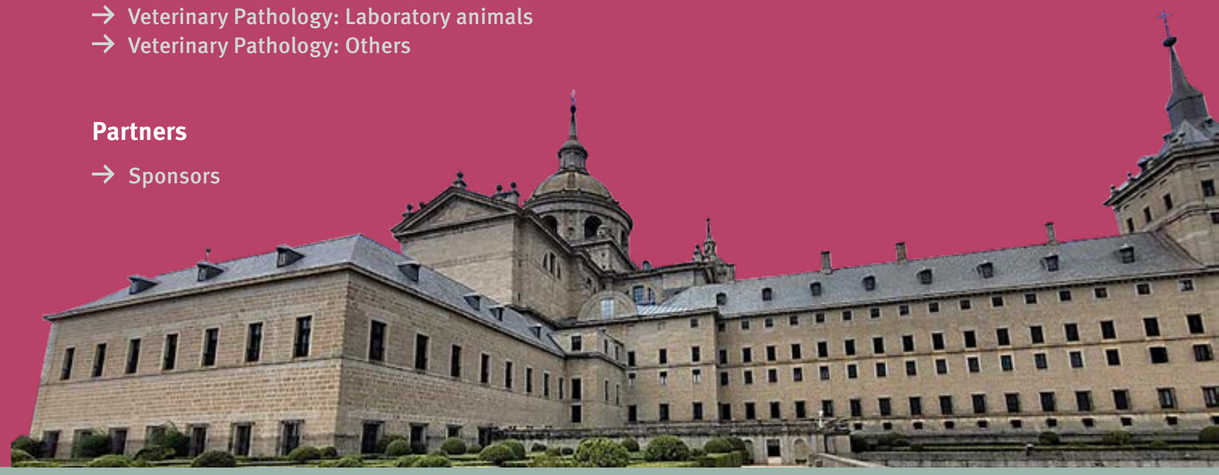

23-26 SEPTEMBER 2025

MANCHESTER, UK

2nd Joint Congress of ESTP and BSTP

CARCINOGENICITY IN THE 21ST CENTURY

CHALLENGES OF NEW METHODOLOGIES, STRATEGIES, AND THEIR IMPACT ON PATHOLOGISTS

BSTP

European Society of Toxicologic Pathology

estp

SAVE THE DATE | FOR MORE INFORMATION VISIT

www.estp-congress.eu

TURIN 2025
ANNUAL ESVP-ECVP CONGRESS

27-30 AUGUST 2025
TURIN - ITALY
SAVE THE DATE

European Society of Veterinary Pathology

European College of Veterinary Pathologists

www.esvp-ecvp-congress.eu

LOCAL ORGANIZING COMMITTEE, SCIENTIFIC ORGANIZING COMMITTEE AND CONGRESS ORGANIZERS

Local Organizing Committee

Laura Peña Fernández (co-chair)
Juana María Flores Landeira (co-chair)
Angela Alonso Diez
Lucia Barreno San Antolín
Jimena De Andrés Gamazo
Virginia Gamino Rodríguez
Rosa García Fernández
Pilar García Palencia
Marta González Huecas
Ma Ángeles Jiménez Martínez
Belén Sánchez Maldonado
Manuel Pizarro Díaz
Antonio Rodríguez Bertos
Ma Ángeles Sánchez Pérez
Enrique Tabanera De Lucio
Edgar Guillermo Valdivia Lara

Scientific Organizing Committee - ESTP

Anna-Lena Frisk
Alison Rowles
Alok Sharma
Babunilayam Gangadharan
Begonya Garcia
Brad Bolon
Davide Corbetta
Deepa Rao
Diethilde Juliane Theil
Elizabeth Galbreath
Emily Meseck
Ingrid Pardo
Kerstin Hahn
Martina Stirn
Melissa Czajkowski
Pierre Maliver
Peter Maslej
Sibylle Groeters
Stefanie Arms
Sameh Youssef
Venkatesha Udupa
Wolfgang Kaufmann
Xavier Palazzi

Scientific Organizing Committee - ESVP/ECVP

Laura Peña
Pompei Bolfa
Elena Riccardi
Luca Aresu
Pedro Faisca
Anna Oevermann
Andrea Gröne

Representatives IATP Symposium

Ramesh Kovi
Deepa Rao
Sibylle Groeters

Congress Organizers

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Avenue Ceramique 222, 6221 KX Maastricht,
The Netherlands
Contact person: Mrs. Mirel Kostons
m.kostons@pcopartners.nl
T +31(0)43 321 84 80

pco PARTNERS IN CONGRESS ORGANISATION

USEFUL INFORMATION

The CEP Congress will be organized at:
Real Colegio Universitario Maria Cristina
Paseo de Los Alamillos 2, 28200 San Lorenzo de El Escorial (Madrid)

Real Colegio Universitario Maria Cristina

Nowadays, the venue is an upper educational center associated with the complutense University of Madrid. It is an unique university campus, with an outstanding atmosphere and architecture. Going back to it's origin, the construction work was undertaken between 1590 and 1597, with the aim of housing in a single building all the warehousing, services and animals of the Hieronymite Community, the religious order entrusted with the care and maintenance of the Monastery.

Registration Desk

The registration area in the conference centre will be open for registration and questions on:

Wednesday 28 August: **08.00-19.00**
Thursday 29 August: **07.30-18.00**
Friday 30 August: **07.30-18.00**
Saturday 31 August: **08.30-13.00**

Please note that the official currency at the congress is the Euro.
At the registration desk cash, cheques and foreign currency are not accepted.

The registration fee includes

- **Admission to all scientific sessions**
- **Lunch Wednesday, Thursday and Friday**
- **Final programme**
- **Congress Dinner**
- **Admission to the exhibition area**
- **Daily coffee breaks**
- **Welcome reception**

WIFI

You will have free WIFI access on-site in the congress venue
Network: **EVENTOS**
Password: **Maria Cristina**

Congress badges

All participants, speakers and exhibitors must wear the identification badges.
Entrance to meeting halls and exhibition area will not be permitted to any person without badge.

Certificate of attendance

A certificate of attendance can be downloaded after submission of the online evaluation which will be requested to be filled in after the congress. You will receive an invitation via email after the congress.

Interactive Slides

Some speakers will incorporate interactive slides into their presentation. A smart phone is required to participate in the voting.

Poster Presentations

Posters will be exhibited on Thursday and Friday in the hallways around the courtyard. Authors are kindly requested to be at their posters during the breaks to answer potential questions.

Anything lost?

Please go to the registration desk.

Language

The official language of the congress is English.

Mobile phones

Please silence your mobile phones during the lectures.

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.

CITYMAP

Gala Dinner

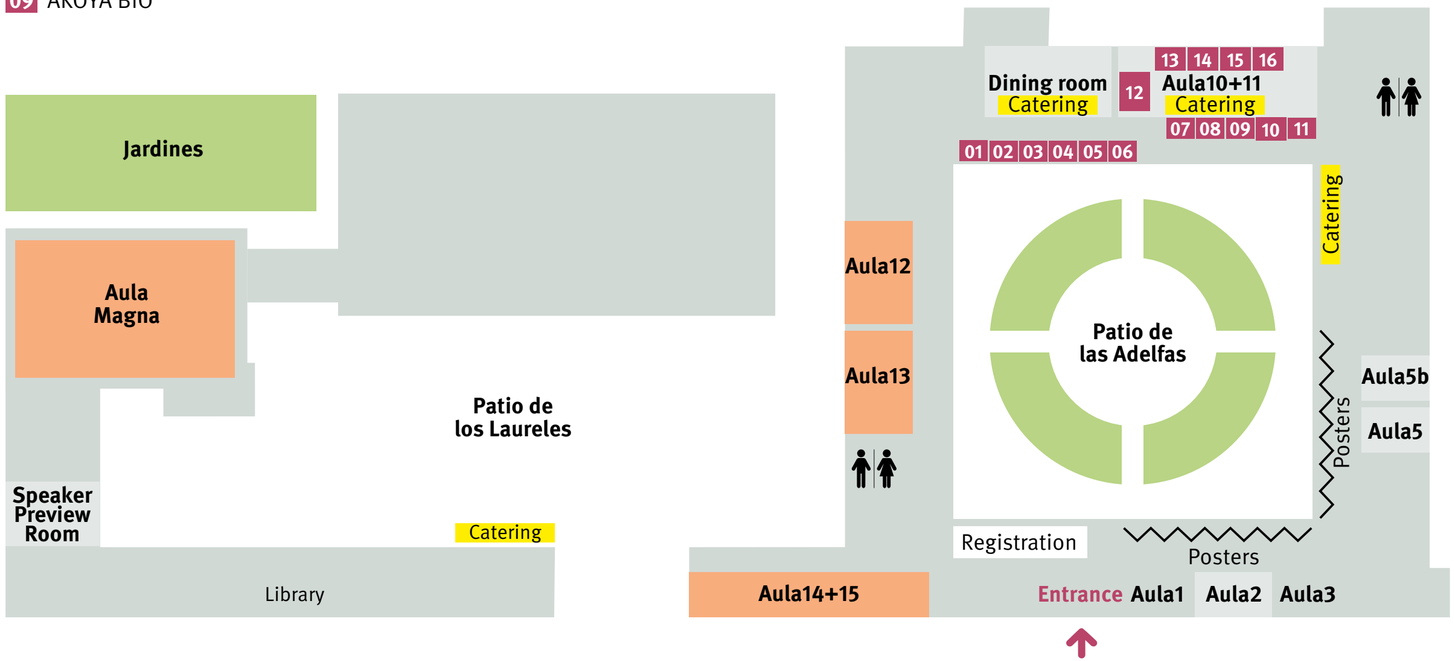
Finca Miravalle
Carretera de A Coruña,
28440 Guadarrama

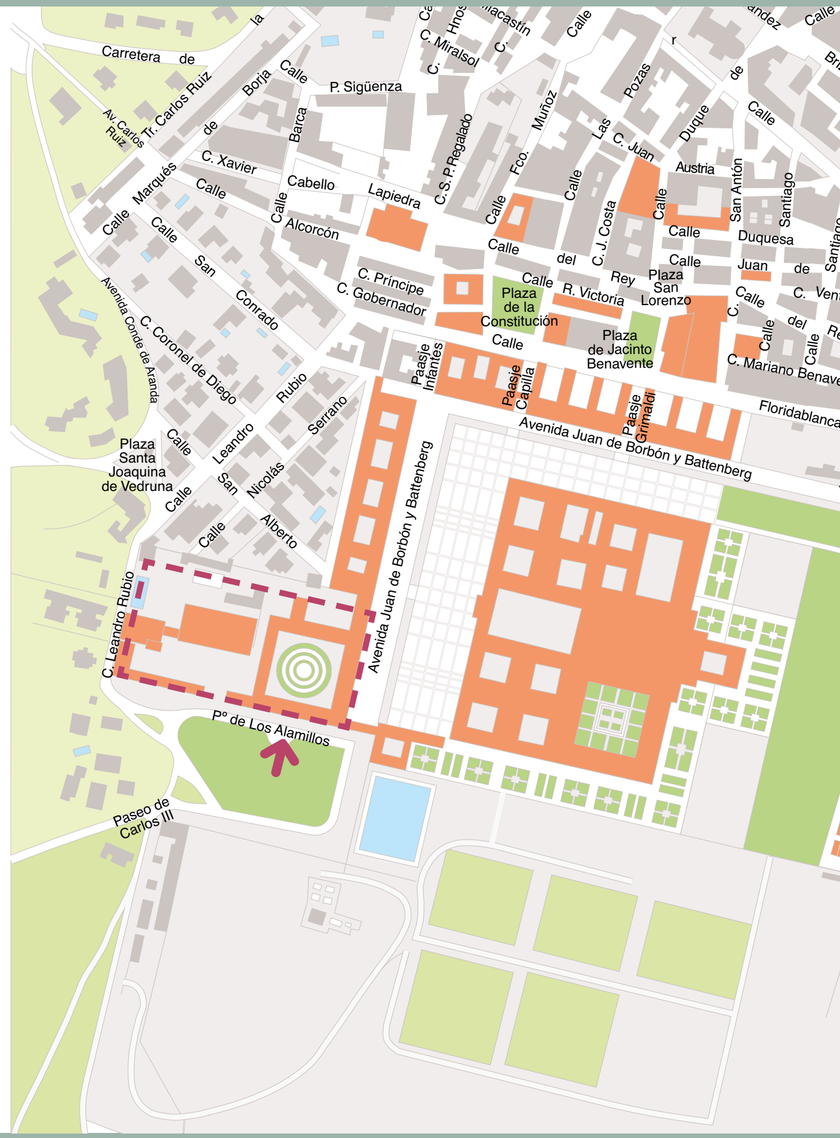
Shuttle busses will be provided from and to San Lorenzo de El Escorial, Sercotel Gran Hotel Conde Duque and Hotel Exe Moncloa Madrid

EXHIBITION FLOORPLAN

01 STANDARD BIO
02 PROSCIA
03 IDEXX
04 RESERO ANALYTICS
05 DECIPHEX
06 SMART IN MEDIA
07 AIRAMATRIX
08 SLIDE SCORE
09 AKOYA BIO
10 AIFORIA
11 HAMMAMTSU
12 3D HISTECH
13 INDICALABS
14 INSTEM
15 EVIDENT
16 ERBC

REAL CENTRO UNIVERSITARIO
ESCORIAL - MARÍA CRISTINA





PROGRAMME | WEDNESDAY 28TH AUGUST 2024

IATP Sessions | Resident Day Sessions | ESTP Sessions
ESVP/ECVP Sessions | Joint Plenary Sessions

AULA MAGNA | ESTP Session	AULA 14+15 | Resident Day	AULA 13 | IATP Symposium
	08.30-10.30 Analysis of single cells to boost your research in pathology-Part I *Sven Rottenberg* **OMICS**	**09.00-09.10** Welcome and introduction *IATP Education Committee* **09.10-09.55** Scientific Efforts for Gaining Regulatory Confidence in NAMs for Neurotoxicity *Dr. Ellen Fritsche* **09.55-10.40** Current State of Developmental Neurotoxicity (DNT) Assesment Using New Approach Methodologies (NAMs) *Dr. Helena Hogberg-Durdock*
	10.30-11.00 COFFEE BREAK	**10.40-11.10 COFFEE BREAK**
	11.00-13.00 Analysis of single cells to boost your research in pathology-Part II *Philipp Olias* **OMICS**	**11.10-11.50** Larval zebrafish as an Alternative in vivo Model for Assessing Seizure Liability: from Behaviour through to Functional Brain Imaging. *Dr. Matthew Winter* **11.50-12.30** Assessment of Chronic Neurotoxicity using NAMs *Dr. Andrea Terron* **12.30-12.55** Panel Discussion **12.55-13.00** Closing Remarks *IATP Education Committee*
	13.00-14.00 LUNCH & REGISTRATION	
Welcome ESTP Chair *Silvia Guionaud* **14.15-14.55** Sampling the Nervous System For Histopathology Evaluation: Getting Aligned to Detect Neurotoxicity *Deepa Bandi Rao* **NEUROMETHODS** *Moderators: Ingrid Pardo & Alok Sharma* **14.55-15.20** Brain and spinal cord sampling for molecular and protein analyses *Xavier Palazzi* **NEUROMETHODS** *Moderators: Ingrid Pardo & Alok Sharma* **15.20-15.45** Direct Drug Delivery Into Brain Parenchyma in Monkeys: Technical Aspects and Pathology Findings *Alexandra Duetting & Annette Romeike* **NEUROMETHODS** *Moderators: Ingrid Pardo & Alok Sharma* **15.45-15.55** Silver staining in neuropathology and neurotoxicity: practical approaches, advantages, and disadvantages *Kristel Kegler* **NEUROMETHODS** *Moderators: Ingrid Pardo & Alok Sharma* **15.55-16.05** Immunohistochemical Characterization of Glial cells Around Holes in the Brain: Two Similar Experiences in the Dog *Stefanie Arms & Enrico Vezzali* **NEUROMETHODS** *Moderators: Ingrid Pardo & Alok Sharma* **16.05-16.15** Myelin methods *Brad Bolon* **NEUROMETHODS** *Moderators: Ingrid Pardo & Alok Sharma*	**14.00-16.00** Pictures of Publication Quality, the perspective of the Photo Editors at the Veterinary Pathology Journal *Silvia Ferro & Hanna Bender* **PRACTICAL SKILLS**	
16.15-16.45 COFFEE BREAK	**16.00-16.30 COFFEE BREAK**	
17.00-17.25 Finding One's Way in the Nervous System Maze with an AI Compass *Erio Barale-Thomas* **NEUROMETHODS** *Moderators: Ingrid Pardo & Alok Sharma* **17.25-18.00** Audience Participation *Moderators: Ingrid Pardo & Alok Sharma* **NEUROMETHODS**	**16.30-18.30** Dealing with residents' challenges *Chiara Palmieri & Pompei Bolfa* **PRACTICAL SKILLS**	
	19.00-21.30 CONGRESS OPENING & WELCOME RECEPTION **Jardines**	

ESVP/ECVP Sessions | Joint Plenary Sessions | ESTP Sessions | Workshop

PROGRAMME | THURSDAY 29TH AUGUST 2024

AULA MAGNA	AULA 14+15	AULA 13	AULA 12
08.15-08.30 Resero Anayltics Sponsored Session Harnessing AI: TurboToxicology Integrates Cutting-Edge LLMs for Enhanced Reporting of Pathology and Toxicology Study Data *David Watson*			
08.30-09.30 The role of the brain barriers in CNS immunity during health and neuroinflammation *Britta Engelhardt* GENERAL PATHOLOGY *Moderators: Anna Oevermann & Elizabeth Galbreath*			
09.30-10.30 Poster session *Moderators: Diethilde Theil & Ingrid Pardo*	09.45-10.30 Poster Flash Presentations *Moderator: Rute Noiva*		
10.30-11.00 COFFEE BREAK | POSTER TOUR & EXHIBITION Johnson & Johnson			
11.00-13.00 Veterinary Dermatopathology *Verena Affolter & Charles Bradley* DIAGNOSTICS-COMPANION ANIMALS *Moderators: Paola Roccabianca & Richard Fox*	11.00-11.45 Performance of Biomarkers NF-L, NSE, Tau and GFAP in Blood and Cerebrospinal Fluid in Rat for the Detection of Nervous System Injury *Warren Glaab* CNS EVALUATION *Moderators: Diethilde Theil & Ingrid Pardo*	11.00-12.00 TB in captive and free-ranging wild animals *Christian Gortazar* WILDLIFE *Moderators: Antonio Fernandez & Judith van den Brand*	
	11.45-12.30 Behavioral tests in drug safety assessment-are they relevant for pathologists? *Andrea Greiter-Wilke* CNS EVALUATION *Moderators: Diethilde Theil & Ingrid Pardo*	12.00-13.00 Oral Presentations Exotic, Wildlife & Zoo Animals *Moderators: Antonio Fernandez & Judith van den Brand*	
	12.30-13.00 AWARDS CEREMONY *Moderators: Diethilde Theil & Ingrid Pardo*		
13.00-14.15 LUNCH | POSTER TOUR & EXHIBITION			
13.45-14.15 Deciphex Sponsored Session Foresight: Enhancing Toxicologic Pathology with AI decision support - through pathologists insights *Laoise Lord Bissett & Lise Bertrand*			
14.15-15.00 Neuropathology through the lens of translational imaging: Opportunities for preclinical therapy assessment *Nicolau Beckmann* CNS EVALUATION *Moderators: Diethilde Theil & Emily Meseck*	14.15-15.45 ORAL PRESENTATIONS DIAGNOSTICS *Moderators: Dimitra Psallas & Valentina Zapulli*	14.15-15.00 Invertebrate Pathology: Approach to Diagnostics and Common Diseases, including Inflammation, Infection, Environmental Stress/Toxin Exposure, Negative Energy Balance, and Neoplasia *Elise LaDouceur* UNUSUAL SPECIES *Moderators: Pompei Bolfa & Udo Hetzel*	14.45-15.45 Diversity, Equity, Inclusion: problem-solving together INTERACTIVE INFORMATION SESSION *Moderators: Chiara Palmieri & Erika Abbondati & Emilian Malek & Sarah Kirschner-Kitz*
15.00-15.45 Electrophysiology (EEG/NCV) *Veronika Stein* CNS EVALUATION *Moderators: Diethilde Theil & Emily Meseck*		15.00-15.45 Overview of transmissible cancers in bivalve molluscs *Antonio Villalba* UNUSUAL SPECIES / ONCOLOGY *Moderators: Pompei Bolfa & Udo Hetzel*	
15.45-16.15 COFFEE BREAK | POSTER TOUR & EXHIBITION Johnson & Johnson			
16.15-17.00 MRI and histhopathology in multiple sclerosis *Christina Granziera* CNS EVALUATION *Moderators: Diethilde Theil & Emily Meseck*	16.15-17.00 Teaching Veterinary Pathology to Veterinary Medicine Students: what European perspective? *Massimo Castagnaro* TEACHING *Moderators: Laura Peña & Andrea Gudan*	16.15-17.45 Oral Presentations Livestock & Laboratory Animals *Moderators: Ingo Gerhauser & Hanne Jahns*	16.15-17.45 Overview of the Histology of Invertebrate Species *Elise LaDouceur* INTERACTIVE WORKSHOP
17.00-17.45 CNS safety biomarker and their application in clinical settings *Tobias Derfuss* CNS EVALUATION *Moderators: Diethilde Theil & Emily Meseck*	17.00-17.45 Oral Presentations Livestock & Others *Moderators: Laura Peña & Andrea Gudan*		
17.45-18.00 You've thought of it, but have you ever seen it?-Interesting neuropathological findings in the brains of Wistar rats *Kathrin Becker* CASE PRESENTATION *Moderators: Diethilde Theil & Emily Meseck*			
	18.00-19.30 ECVP AGM		
18.00-20.00 AIRA MATRIX AIRATOX EUROPEAN LAUNCH Monte De Los Ángeles		19.30-21.00 WISDOM Train your brain	

ESVP/ECVP Sessions | Joint Plenary Sessions | ESTP Sessions | Workshop

PROGRAMME | FRIDAY 30TH AUGUST 2024

AULA MAGNA	AULA 14+15	AULA 13	AULA 12
08.15-08.30 Standard BioTools Sponsored session: Fast high-plex tumor microenvironment investigation: from tissue heterogeneity to single cell analysis *David Guet*			
08.30-09.30 A Fandango of Form and Function: The Interplay between Clinical Neurology and Neuropathology Investigation *Brad Bolon* **GENERAL PATHOLOGY** *Moderators: Pompei Bolfa & Sameh Youssef*			
09.30-10.00 Bridging the Gap: Understanding Dementia through Animal Models *Laura Fusaro* **NEURODEGENERATIVE DISEASES** *Moderators: Alok Sharma & Sameh Youssef*	**09.45-10.30** Poster Flash Presentations *Moderator: Nermin Caliskan*		
10.00-10.30 Autoimmune Neurodegenerative Diseases: Shifting Paradigms and Emerging Opportunities (MS, Guillain-Barre) *Dinesh Bangari* **NEURODEGENERATIVE DISEASES** *Moderators: Alok Sharma & Sameh Youssef*			
10.30-11.00 COFFEE BREAK | POSTER TOUR & EXHIBITION			
11.00-11.45 Using advanced human in vitro models to study neurodegenerative disease *Jeroen Pasterkamp* **NEURODEGENERATIVE DISEASES** *Moderators: Alok Sharma & Sameh Youssef*	**11.00-12.00** Tumours of the urogenital system: the old, the new and the fugitive! *Chiara Palmieri & Valeria Grieco* **DIAGNOSTICS COMPANION ANIMALS** *Moderators: Giancarlo Avallone & Jerome Abadie*	**11.00-12.00** Highly infectious diseases in ruminants - just the usual suspects? *Angele Breithaupt* **FARM ANIMAL HEALTH** *Moderators: Reinie Dijkman & Henrik-Elvang Jensen*	
11.45-12.30 Update on cannabinoid-based therapeutic options for treating neurodegenerative disorders *Javier Ruiz* **NEURODEGENERATIVE DISEASES** *Moderators: Alok Sharma & Sameh Youssef*	**12.00-13.00** Oral Presentations Small Animal *Moderators: Giancarlo Avallone & Jerome Abadie*	**12.00-13.00** Epizootic hemorrhagic disease: an emerging vector-borne viral pathology with a big impact on Spanish cattle *Marcelo de las Heras* **FARM ANIMAL HEALTH** *Moderators: Reinie Dijkman & Henrik-Elvang Jensen*	
12.30-13.00 Panel Discussion *Laura Fusaro & Dinesh Bangari & Jeroen Pasterkamp & Javier Fernández Ruiz* **NEURODEGENERATIVE DISEASES** *Moderators: Alok Sharma & Sameh Youssef*			
13.00-14.15 LUNCH | POSTER TOUR & EXHIBITION			
13.45-14.15 Aira Matrix Sponsored Session: Deep Learning-Based Spermatogenic Staging in Tissue Sections of Cynomolgus Macaque Testes *Svenja Hartung & Chaith Kondragunta*			
14.15-15.15 Towards standardization of histologic tumor prognostic parameters - does it really matter *Christof Bertram & Pompei Bolfa* **ONCOLOGY** *Moderators: Koen Chiers & Simone de Brot*	**14.15-14.35** Past current and future of Developmental Neurotoxicity studies *Frédèric Schorsch* **INTRODUCTION TO DNT** *Moderators: Deepa Bandi Rao & Sibylle Gröters*	**14.15-15.45** Oral Presentations Livestock, Horses, Exotic, Wildlife & Zoo Animals *Moderators: Marian Tailescu & Cecilia Ley*	
	14.35-15.00 Developmental Neuropathology: Introduction and overview *Wolfgang Kaufmann* **DNT** *Moderators: Deepa Bandi Rao & Sibylle Gröters*		
15.15-15.45 Oral Presentations Small Animal & Others *Moderators: Koen Chiers & Simone de Brot*	**15.00-15.45** Morphometrics in developmental neurotoxicity studies- pitfalls and interpretation *Heike Marxfeld* **DNT** *Moderators: Deepa Bandi Rao & Sibylle Gröters*		
15.45-16.15 COFFEE BREAK | POSTER TOUR & EXHIBITION			
16.15-17.15 Humans Forensics -Brain Trauma *Joao Pinheiro* **FORENSICS** *Moderators: Lorenzo Ressel & Pedro Faisca*	**16.15-16.45** Thyroid Hormone Imbalances-Tox Aspects *Stephanie Melching-Kollmus* **DNT** *Moderators: Deepa Bandi Rao & Sibylle Gröters*	**16.15-17.45** How to be better prepared for Data analysis and Tox path problems in the Comprehensive section *Edouard Reyes-Gomez* **TEACHING / ECVP**	**16.15-17.15** Establishing Consistency in Tumor Prognosis Assessment: How do your evaluations stack up against those of your peers? *Christof Bertram & Pompei Bolfa* **INTERACTIVE WORKSHOP**
17.15-18.00 Oral presentations Forensics *Moderators: Lorenzo Ressel & Pedro Faisca*	**16.45-17.15** Thyroid hormone imbalance in pregnant rats & its impact on neurodevelopment in the pups: A hunt for a reliable histopathological biomarker *Babunilayam Gangadharan* **DNT** *Moderators: Deepa Bandi Rao & Sibylle Gröters*		
	17.15-18.45 SEAPV AGM		
		18.00-19.00 ESVP AGM	
19.30-01.00 GALA DINNER | MIRAVALLE			

ESVP/ECVP Sessions | Joint Plenary Sessions | ESTP Sessions | Workshop

PROGRAMME | SATURDAY 31ST AUGUST 2024

AULA MAGNA	AULA 14+15	AULA 13	AULA 12
	09.00-09.45 **Computational pathology applied to toxicologic pathology: learning to ride AI's wave of opportunity** *Julie Boisclair* **RECENT NEURO CONCEPTS** *Moderators: Pierre Maliver & Elizabeth Galbreath*		
09.30-11.00 **Whats'your Diagnosis** *Pedro Faísca & Luca Aresu* **INTERACTIVE MYSTERY SLIDE SESSION** smart in media intelligent software	**09.45-10.00** **Digital pathology and AI applied to tox path: Q&A-free discussion** **RECENT NEURO CONCEPTS** *Pierre Maliver, Elizabeth Galbreath, Julie Boisclair & Erio Barale-Thomas*		
	10.00-10.45 **Neuropathology insights via cryofluorescent tomography (CFT)** *Elizabeth Galbreath* **RECENT NEURO CONCEPTS** *Moderators: Pierre Maliver & Elizabeth Galbreath*		
	10.45-11.00 **Case Presentation: Comparative AAV-duplex amiR-SOD1 -related findings in nervous system of Cynomolgus monkeys, New Zealand White rabbits, and C57BL/6 mice** *Ingrid Pardo* *Moderators: Pierre Maliver & Elizabeth Galbreath*		
11.00-11.30 COFFEE BREAK			
11.30-12.15 **Tissue technology in blood-brain barrier organoids as combined efficacy/toxicity high throughput spatial readouts in early drug screening** *Luisa Bell* **RECENT NEURO CONCEPTS** *Moderators: Pierre Maliver & Elizabeth Galbreath*	**11.30-12.15** **PARR what does it mean?***Manfred Henrich* **DIAGNOSTICS** *Moderators: Elena Riccardi & Christof Bertram*	**11.30-13.00** **The Global Health Pathology Network (GHPN) and Establishing Relationships Through Foundational Pathology Training: An Information Session** *Javier Asin* **INFORMATION SESSION**	**11.30-13.00** **Certificate in Veterinary Forensic Pathology: a Simulation** *SteeringGroup* **INTERACTIVE WORKSHOP FORENSIC**
12.15-13.00 **Unveiling CNS Complexity and Drug Responses through Spatial Transcriptomics** *Kerstin Hahn & Benedek Pesti* **RECENT NEURO CONCEPTS** *Moderators: Pierre Maliver & Elizabeth Galbreath*	**12.15-13.00** **From Pixels to Patterns: The Transformative Power of AI in Neuropathology Research** *Lev Stimmer* *Moderators: Elena Riccardi & Christof Bertram*		
13.00-13.15 **Case Presentation: Bilateral basal nuclei vacuolar lesions-A novel and emerging potential background finding in Beagle dogs** *Stefanie Arms* *Moderators: Pierre Maliver & Elizabeth Galbreath*			
13.15-13.30 **Case Presentation: Ultrastructural changes in sensory nerves: Findings in DRG and peripheral nerves** *Emily Meseck* *Moderators: Pierre Maliver & Elizabeth Galbreath*			

SOCIAL PROGRAM

Wednesday 28th August 2024 | Congress Opening and Welcome Reception

19.00-20.00 | Congress Opening
20.00-21.30 | Welcome Reception

Venue: Real Colegio Universitario Maria Cristina
The CEP Congress invites all attendees to join during the official congress opening! The welcome reception is a great opportunity to network, meet old friends and colleagues, and make new ones. We will start the congress with an official welcome, followed by a traditional Spanish entertainment performance. After the official opening you can enjoy a 'Spanish Wine' in the courtyard of Real Colegio Universitario Maria Cristina , with some drinks and canapes.

Friday 30th August 2024 | Gala Dinner & Dance Party

19.30-01.00 | Gala Dinner & Dance Party

Venue: Finca Miravalle, Carretera de A Coruña, 28440 Guadarrama Madrid*
* * Shuttle busses will be arranged from and to San Lorenzo de El Escorial and back to Sercotel Gran Hotel Conde Duque and Hotel Exe Moncloa.

The Gala Dinner will take place at the beautiful Finca Miravalle, located just outside San Lorenzo de El Escorial. Surrounded by more than 5,000 square meters of gardens, creating the perfect backdrop for a wonderful evening. The evening will start with an aperitif in the gardens, followed by a seated dinner. We are ending the evening with a dance party with a live DJ!

SIDE MEETINGS ESTP

Wednesday 28th August 2024 | Aula 2, 15.30-16.00
ESTP DigPath committee | Informal gathering
Chair: Lise Bertrand

Thursday 29th August 2024 | Aula 2, 11.35-12.35
Early Career Meeting | Open meeting

Thursday 29th August 2024 | Aula 2, 13.15-14.15
SOC 2025 Meeting | Invitation only
Chair: Thomas Nolte

SIDE MEETINGS ESVP/ ECVP

Wednesday 28th August 2024 | Aula 1, 09.00-18.00
ESVP Board Meeting | On invitation only
Chair: Jérôme Abadie

Wednesday 28th August 2024 | Aula 3, 08.00-18.30
ECVP Board Meeting | On invitation only
Chair: Alexandre Bidaut

Wednesday 28th August 2024 | Aula 5, 14.00-17.00
CFVP Round table | On invitation only
Chair: Lorenzo Ressel

SPEAKER ABSTRACTS | WEDNESDAY 28 AUGUST 2024

Resident Day

14.00-16.00 PICTURES OF PUBLICATION QUALITY, THE PERSPECTIVE OF THE PHOTO EDITORS AT THE VETERINARY PATHOLOGY JOURNAL *Silvia Ferro*

16.30-18.30 DEALING WITH RESIDENT'S CHALLENGES *Chiara Palmieri & Pompei Bolfa*

IATP Symposium

09.10-09.55 SCIENTIFIC EFFORTS FOR GAINING REGULATORY CONFIDENCE IN NAMS FOR NEUROTOXICITY *Ellen Fritsche*

09.55-10.40 CURRENT STATE OF DEVELOPMENTAL NEUROTOXICITY (DNT) ASSESSMENT USING NEW APPROACH METHODOLOGIES (NAMS) *Helena Hogberg-Durdock*

11.10-11.50 LARVAL ZEBRAFISH AS AN ALTERNATIVE IN VIVO MODEL FOR ASSESSING SEIZURE LIABILITY: FROM BEHAVIOUR THROUGH TO FUNCTIONAL BRAIN IMAGING *Matthew Winter*

11.50-12.30 ASSESSMENT OF CHRONIC NEUROTOXICITY USING NAMS *Andrea Terron*

Toxicological Pathology

14.15-14.55 SAMPLING THE NERVOUS SYSTEM FOR HISTOPATHOLOGY EVALUATION: GETTING ALIGNED TO DETECT EUROTOXICITY *Deepa Bandi Rao*

14.55-15.20 BRAIN AND SPINAL CORD SAMPLING FOR MOLECULAR AND PROTEIN ANALYSIS *Xavier Palazzi*

15.20-15.45 DIRECT DRUG DELIVERY INTO BRAIN PARENCHYMA IN MONKEYS: TECHNICAL ASPECTS AND PATHOLOGY FINDINGS *Alexandra Duetting & Annette Romeike*

15.45-15.55 SILVER STAINING IN NEUROPATHOLOGY AND NEUROTOXICITY: PRACTICAL APPROACHES, ADVANTAGES, AND DISADVANTAGES *Kristel Kegler*

15.55-16.05 IMMUNOHISTOCHEMISTRY CHARACTERIZATION OF GLIAL CELLS AROUND "HOLES" IN THE BRAIN - TWO SIMILAR EXPERIENCES IN THE DOG *Stefanie Arms & Enrico Vezzali*

16.05-16.15 MYELIN METHODS *Brad Bolon*

17.00-17.25 FINDING ONE'S WAY IN THE NERVOUS SYSTEM MAZE WITH AN AI COMPASS *Erio Barale-Thomas*

SPEAKER ABSTRACTS | THURSDAY 29 AUGUST 2024

Joint Stream

08.30-09.30 THE ROLE OF THE BRAIN BARRIERS IN CNS IMMUNITY DURING HEALTH AND NEUROINFLAMMATION *Britta Engelhardt*

Toxicological Pathology

11.00-11.45 PERFORMANCE OF BIOMARKERS NF-L, NSE, TAU AND GFAP IN BLOOD AND CEREBROSPINAL FLUID IN RAT FOR THE DETECTION OF NERVOUS SYSTEM INJURY *Warren Glaab*

11.45-12.30 BEHAVIORAL TESTS IN DRUG SAFETY ASSESSMENT - ARE THEY RELEVANT FOR PATHOLOGISTS? *Andrea Greiter-Wilke*

14.15-15.00 NEUROPATHOLOGY THROUGH THE LENS OF TRANSLATIONAL IMAGING: OPPORTUNITIES FOR PRECLINICAL THERAPY ASSESSMENT *Nicolau Beckmann*

15.00-15.45 ELECTROPHYSIOLOGY (EMG/NCV) *Veronika Stein*

16.15-17.00 MRI AND HISTHOPATHOLOGY IN MULTIPLE SCLEROSIS *Christina Granziera*

17.00-17.45 CNS SAFETY BIOMARKER AND THEIR APPLICATION IN CLINICAL SETTINGS *Tobias Derfuss*

Veterinary Pathology

11.00-13.00 VETERINARY DERMATOPATHOLOGY *Verena Affolter & Charles Bradley*

11.00-12.00 TB IN CAPTIVE AND FREE-RANGING WILD ANIMALS *Christian Gortazar*

14.15-15.00 INVERTEBRATE PATHOLOGY: APPROACH TO DIAGNOSTICS AND COMMON DISEASES, INCLUDING INFLAMMATION, INFECTION, ENVIRONMENTAL STRESS/TOXIN EXPOSURE, NEGATIVE ENERGY BALANCE, AND NEOPLASIA *Elise LaDouceur*

15.00-15.45 OVERVIEW OF TRANSMISSIBLE CANCERS IN BIVALVE MOLLUSCS *Antonio Villalba*

16.15-17.00 TEACHING VETERINARY PATHOLOGY TO VETERINARY MEDICINE STUDENTS: WHAT EUROPEAN PERSPECTIVE? *Massimo Castagnaro*

SPEAKER ABSTRACTS | FRIDAY 30 AUGUST 2024

Joint Stream

08.30-09.30 A FANDANGO OF FORM AND FUNCTION: THE INTERPLAY BETWEEN CLINICAL NEUROLOGY AND NEUROPATHOLOGY INVESTIGATION *Brad Bolon*

Toxicological Pathology

09.30-10.00 BRIDGING THE GAP: UNDERSTANDING DEMENTIA THROUGH ANIMAL MODELS *Laura Fusaro*
10.00-10.30 AUTOIMMUNE NEURODEGENERATIVE DISEASES: SHIFTING PARADIGMS AND EMERGING OPPORTUNITIES *Dinesh Bangari*
11.00-11.45 USING ADVANCED HUMAN IN VITRO MODELS TO STUDY NEURODEGENERATIVE DISEASE *Jeroen Pasterkamp*
11.45-12.30 UPDATE ON CANNABINOID-BASED THERAPEUTIC OPTIONS FOR TREATING NEURODEGENERATIVE DISORDERS *Javier Fernández Ruiz*
14.15-14.35 PAST CURRENT AND FUTURE OF DEVELOPMENTAL NEUROTOXICITY STUDIES *Frédèric Schorsch*
14.35-15.00 DEVELOPMENTAL NEUROPATHOLOGY: INTRODUCTION AND OVERVIEW *Wolfgang Kaufmann*
15.00-15.45 MORPHOMETRICS IN DEVELOPMENTAL NEUROTOXICITY STUDIES- PITFALLS AND INTERPRETATION *Heike Marxfeld*
16.15-16.45 THYROID HORMONE IMBALANCES - TOX ASPECTS *Stephanie Melching-Kollmus*
16.45-17.15 THYROID HORMONE IMBALANCE IN PREGNANT RATS AND ITS IMPACT ON NEURODEVELOPMENT IN THE PUPS: A HUNT FOR A RELIABLE HISTOPATHOLOGICAL BIOMARKER *Babunilayam Gangadharan*

Veterinary Pathology

11.00-12.00 TUMOURS OF THE UROGENITAL SYSTEM: THE OLD, THE NEW AND THE FUGITIVE! *Chiara Palmieri & Valeria Grieco*
11.00-12.00 HIGHLY INFECTIOUS DISEASES IN RUMINANTS-JUST THE USUAL SUSPECTS? *Angele Breithaupt*
12.00-13.00 EPIZOOTIC HEMORRHAGIC DISEASE: AN EMERGING VECTOR-BORNE VIRAL PATHOLOGY WITH A BIG IMPACT ON SPANISH CATTLE *Marcelo de las Heras*
14.15-15.15 TOWARDS STANDARDIZATION OF HISTOLOGIC TUMOR PROGNOSTIC PARAMETERS - DOES IT REALLY MATTER? C*hristof Bertram & Pompei Bolfa*
16.15-17.15 HUMANS FORENSICS -BRAIN TRAUMA *Joao Pinheiro*

SPEAKER ABSTRACTS | SATURDAY 31 AUGUST 2024

Toxicological Pathology

09.00-09.45 COMPUTATIONAL PATHOLOGY APPLIED TO TOXICOLOGIC PATHOLOGY: LEARNING TO RIDE AI'S WAVE OF OPPORTUNITY *Julie Boisclair*

10.00-10.45 NEUROPATHOLOGY INSIGHTS VIA CRYOFLUORESCENT TOMOGRAPHY (CFT) *Elizabeth Galbreath*

11.30-12.15 TISSUE TECHNOLOGY IN BLOOD-BRAIN BARRIER ORGANOIDS AS COMBINED EFFICACY/TOXICITY HIGH THROUGHPUT SPATIAL READOUTS IN EARLY DRUG SCREENING *Luisa Bell*

12.15-13.00 UNVEILING CNS COMPLEXITY AND DRUG RESPONSES THROUGH SPATIAL TRANSCRIPTOMICS *Kerstin Hahn & Benedek Pesti*

Veterinary Pathology

11.30-13.00 THE GLOBAL HEALTH PATHOLOGY NETWORK (GHPN) AND ESTABLISHING RELATIONSHIPS THROUGH FOUNDATIONAL PATHOLOGY TRAINING: AN INFORMATION SESSION *Javier Asin*

11.30 -12.15 PARR: WHAT DOES IT MEAN? *Manfred Henrich*

12.15-13.00 FROM PIXELS TO PATTERNS: THE TRANSFORMATIVE POWER OF AI IN NEUROPATHOLOGY RESEARCH *Lev Stimmer*

CASE PRESENTATIONS

Toxicological Pathology | Thursday 29th August 2024

17.45-18.00 YOU'VE THOUGHT OF IT, BUT HAVE YOU EVER SEEN IT? - INTERESTING NEUROPATHOLOGICAL FINDINGS IN THE BRAINS OF WISTAR RATS *Kathrin Becker*

Toxicological Pathology | Saturday 31st August 2024

10.45-11.00 COMPARATIVE AAV-AMIR-SOD1-RELATED FINDINGS IN NERVOUS SYSTEM OF CYNOMOLGUS MONKEYS, NEW ZEALAND WHITE RABBITS, AND C57BL/6 MICE *Ingrid Pardo*

13.00-13.15 BILATERAL BASAL NUCLEI VACUOLAR LESIONS - A NOVEL AND EMERGING POTENTIAL BACKGROUND FINDING IN BEAGLE DOGS *Stefanie Arms*

13.15-13.30 ULTRASTRUCTURAL CHANGES IN SENSORY NERVES: FINDINGS IN DRG AND PERIPHERAL NERVES *Emily Meseck*

POSTER FLASH

Veterinary Pathology | Thursday 29th August 2024

09.45-09.51 EVALUATION OF ALTERNATIVE IMMUNIZATION STRATEGIES AGAINST PARATUBERCULOSIS IN GOATS *Miguel Criado*

09.51-09.57 EXTRACELLULAR VESICLE ISOLATION AND CHARACTERIZATION FROM BOTTLENOSE DOLPHIN'S (TURSIOPS TRUNCATUS) BLOW *Valentina Moccia*

09.57-10.03 METHODOLOGY: MASS SPECTROMETRY-BASED IDENTIFICATION OF ANIMAL AMYLOIDOSIS *Tomoaki Murakami*

10.03-10.09 MULTISYSTEMIC MYXOZOAN INFECTION IN TOADFISH, TETRACTENOS HAMILTONI CAUSED BY MONOMYXUM INCOMPTAVERMI *Arashi Nakashimi*

10.09-10.15 COMPARATIVE ANATOMY AND HISTOLOGY OF GUSTATORY PAPILLAE IN LABORATORY ANIMAL SPECIES *Ricardo de Miguel*

Veterinary Pathology | Friday 30th August 2024

09.45-09.51 FROM CONCEPT TO REALITY: THE JOURNEY OF ESTABLISHING THE AUSTRALIAN COMPANION ANIMAL REGISTRY OF CANCERS (ACARCINOM) *Chiara Palmieri*

09.51-09.57 PORCINE NEPHROBLASTOMA AS A SPONTANEOUS ANIMAL MODEL FOR WILMS'TUMOR: PRELIMINARY RESULTS *Eleonora Brambilla*

09.57-10.03 STAT3 PATHWAY IN CANINE ORAL MELANOMA: A POTENTIAL THERAPEUTIC TARGET? *Adriana Lo Giudice*

10.03-10.15 TILS, TLSS AND HLMS EVALUATION IN CANINE MAMMARY CARCINOMAS *Giada Giambrone*

10.15-10.21 PORCINE ALVEOLAR MACROPHAGES AND NASAL EPITHELIUM CAN INTERNALIZE PORCINE EPIDEMIC DIARRHEA VIRUS (PEDV) BUT DO NOT SUPPORT REPLICATION IN VITRO *Carlos López Figueroa*

10.21-10.27 IMPACT OF HANDLING TECHNIQUES ON UMBILICAL ULCERATIONS AND OMPHALITIS IN PIGLETS *Kristiane Barington*

10.27-10.33 CANINE ORAL MELANOMA MACROPHAGE-POOR HETEROSPHEROIDS: AN UPSIDE DOWN MODEL TO INVESTIGATE TUMOR MICROENVIRONMENT *Ilaria Porcellato*

ORAL ABSTRACTS

Veterinary Pathology | Thursday 29th August 2024

12.00-12.12 THE SKIN I LIVE IN. THE INTRACELLULAR LIFESTYLE OF P. DESTRUCTANS DURING WHITE-NOSE SYNDROME OF BATS *Marcos Isidoro-Ayza*
12.12-12.24 DETECTION OF HIGHLY PATHOGENIC AVIAN INFLUENZA H5N1 IN MARINE MAMMALS FROM SOUTH AMERICA *Eva Sierra*
12.24-12.36 HIGHLY METASTATIC MAMMARY CARCINOMAS IN TWO PATAGONIAN SEA LIONS *Elena Colombino*
12.36-12.48 PATHOLOGIES AND CAUSES OF DEATH IN STRANDED CETACEANS IN THE CANARY ISLANDS (2013-2018) P*ablo Jose Diaz Santana*
12.48-13.00 MORTALITY IN ENDANGERED MAUI DOLPHINS *Wendi Roe*
14.15-14.27 EXPLORING NEW DIAGNOSTIC MARKERS IN FELINE NASAL LYMPHOMA *Rute Noiva*
14.27-14.39 EVIDENCE OF RUSTRELA VIRUS-ASSOCIATED FELINE STAGGERING DISEASE IN SWEDEN SINCE THE 1970S *Emma Thilén*
14.39-14.51 KEY MILESTONES FOR THE DIAGNOSTIC OF EPIZOOTIC HEMORRHAGIC DISEASE IN CALVES *Ana Rodríguez-Largo*
14.51-15.03 BONE PATHOLOGY IN COMMERCIAL PIGS: TIME TO DEAL WITH THE ELEPHANT IN THE ROOM? *Albert Canturri*
15.03-15.15 FROM PIXELS TO PIGLETS: UTILIZING DIGITAL IMAGE ANALYSIS AND DEEP LEARNING TO COMBAT PORCINE DIARRHEA *Cecilie Brandt Becker*
15.15-15.27 HEDGEHOG ARTERIVIRUS-ASSOCIATED ENCEPHALITIS IN CAPTIVE EUROPEAN HEDGEHOGS (ERINACEUS EUROPAEUS) IN WILDLIFE REHABILITATION CENTRES IN ENGLAND *Fabian ZX Lean*
15.27-15.39 COMBINING STEREOLOGY AND IMAGE ANALYSIS FOR ACCURATE AND FAST QUANTIFICATION IN EXPERIMENTAL PATHOLOGY *Pedro Faísca*
16.15-16.27 EFFECT OF THE ORAL ADMINISTRATION OF HEAT-KILLED MYCOBACTERIUM MANRESENSIS ON INTESTINAL HEALTH AND IMMUNOLOGY PARAMETERS OF WEANED PIGLETS C*armen Álvarez-Delgado*
16.27-16.39 LIVER LESIONS IN SHEEP VACCINATED AGAINST F. HEPATICA WITH AND WITHOUT NALTREXONE AS IMMUNE MODULATOR *María Teresa Ruiz-Campillo*
16.39-16.51 EFFECTS OF VACCINATION ON THE IMMUNOHISTOCHEMICAL EXPRESSION OF IFN-Y AND MACROPHAGE POLARIZATION IN CALVES EXPERIMENTALLY INFECTED WITH PARATUBERCULOSIS *David Zapico*
16.51-17.03 EARLY PATHOGENESIS AND INNATE IMMUNE RESPONSES AGAINST TWO STRAINS OF PORCINE EPIDEMIC DIARRHOEA VIRUS IN NEWBORN AND WEANED PIGLETS *Carlos López-Figueroa*
17.03-17.15 ADVERSE REACTIONS AFTER VACCINATION IN SHEEP: A REVIEW OF EXTRINSIC EVENTS OVER THE LAST 30 YEARS *Estela Pérez*
17.15-17.27 DIGITAL HISTOPATHOLOGY ANALYSIS OF LYMPHOID ORGANS FROM GOATS EXPERIMENTALLY INFECTED WITH HIGHLY AND MILDLY VIRULENT PPRV STRAINS *Llorenç Grau-Roma*
17.27-17.39 QUANTITATIVE AND QUALITATIVE ASSESSMENT OF HISTOLOGIC LESIONS IN HONEY BEES (APIS MELLIFERA) INFECTED WITH CHRONIC BEE PARALYSIS VIRUS *Peter Richards-Rio*s
17.00-17.12 SLC25A12-RELATED INHERITED FORM OF PORENCEPHALY IN LIMOUSIN CALVES *Tobias Floyd*
17.12-17.24 DISTINCT SUBCELLULAR DYNAMICS OF LISTERIA MONOCYTOGENES IN MICROGLIA AND MONOCYTE-DERIVED MACROPHAGES: IMPLICATIONS FOR THE PATHOGENESIS OF NEUROLISTERIOSIS *Margherita Polidori*
17.24-17.36 SUSTAINED REDUCTION OF 2-OXOGLUTARATE DEHYDROGENASE COMPLEX ACTIVITY IN RAT BRAIN AFTER VENTRICULAR FIBRILLATION CARDIAC ARREST MAY CONTRIBUTE TO EXTENDED EXCITOTOXICITY *Sandra Högler*

ORAL ABSTRACTS

Veterinary Pathology | Friday 30th August 2024

12.00-12.12 TUMOR INFILTRATING LYMPHOCYTES (TILS) VARIES IN DIFFERENT CANINE SOFT TISSUE SARCOMA (STS) TYPES. *Giancarlo Avallone*

12.12-12.24 ASSOCIATION BETWEEN DOMESTIC CAT HEPADNAVIRUS AND FELINE HEPATOCELLULAR CARCINOMA *Min Chun Chen*

12.24-12.36 PATIENT-DERIVED EXPLANTS FROM CANINE MAMMARY GLAND CANCER USING HETEROLOGOUS PLATELET-RICH PLASMA AS CULTURE MEDIUM *Alessandra Sfacteria*

12.36-12.48 HIGH-THROUGHPUT MULTI-DRUG SCREENING FOR IDENTIFYING POTENTIAL NEW THERAPIES IN MUCOSAL MELANOMA *Alice Musi*

12.48-13.00 FELINE NASAL PLANUM SQUAMOUS CELL CARCINOMA: PRELIMINARY INVESTIGATION ON EPITHELIAL TO MESENCHYMAL TRANSITION (EMT) AND FELINE PAPILLOMA VIRUS INFECTION STATUS *Federico Armando*

13.00-13.12 DIGITAL KARYOMETRY CONFIRMS INCREASED NUCLEAR SIZE AS DIAGNOSTIC TUMOR MARKER FOR CANINE UROTHELIAL CARCINOMA *Simone de Brot*

14.15-14.27 FREQUENCY OF DETECTION OF PORCINE CIRCOVIRUSES IN RETROSPECTIVELY SELECTED CASES OF PORCINE DERMATITIS AND NEPHROPATHY SYNDROME *Àlex Cobos*

14.27-14.39 IDIOPATHIC INTERSTITIAL PNEUMONIAS IN FOALS FROM CALIFORNIA, 1990-2020 *Javier Asin*

14.39-14.51 OVINE ICHTHYOSIS CONGENITA: DESCRIPTION AND CLINICOPATHOLOGIC EVOLUTION *Álex Gómez*

14.51-15.03 ULTRASTRUCTURAL AND TRANSCRIPTOME ANALYSIS OF CEREBELLUM IN PIGLETS BORN WITH CONGENITAL TREMOR TYPE A-II, A LONGITUDINAL STUDY *Anna Bergfeldt*

15.03-15.15 CARPAL CONTRACTURE-A PILOT STUDY OF AN INCREASING FRONT LEG PROBLEM IN NORWEGIAN PIG *Randi Sørby*

15.15-15.27 BLUETONGUE IN BIGHORN SHEEP IN CENTRAL/SOUTHERN CALIFORNIA *Eileen Henderson*

15.27-15.39 PNEUMONIA IN CALIFORNIA DONKEYS *Nicolas Streitenberger*

15.15-15.27 MULTIPLEX IMMUNOHISTOCHEMISTRY AND SUPERVISED MACHINE LEARNING FOR IMMUNE CELLS TOPOGRAPHY IN CANINE PDL1 TESTED UROTHELIAL CARCINOMA *Luisa Vera Muscatello*

15.27-15.39 IMPLEMENTING AI IN DIGITAL HISTOPATHOLOGY: TOWARDS QUANTITATIVE ANALYSIS AND MACHINE LEARNING INTEGRATION IN DIAGNOSTICS AND RESEARCH *Constance De Meeûs d'Argenteuil*

17.15-17.27 USING NEXT-GENERATION SEQUENCING AND IN SITU HYBRIDIZATION TO IDENTIFY THANATOTRANSCRIPTOMIC EXPRESSIONS OF MOUSE BRAINS FOR ESTIMATING POSTMORTEM INTERVALS *Wei-Hsiang Huang*

17.27-17.39 INTESTINAL AND PERITONEAL LESIONS IN WOUNDED UMBILICAL OUTPOUCHINGS IN PIGS AFTER TRANSPORTATION *Henrik Elvang Jensen*

POSTER ABSTRACTS

Toxicological Pathology

POSTER ABSTRACTS

Veterinary Pathology: Livestock

POSTER ABSTRACTS

Veterinary Pathology: Small Animal

POSTER ABSTRACTS

Veterinary Pathology: Horses

POSTER ABSTRACTS

Veterinary Pathology: Exotic, wildlife & zoo animals

POSTER ABSTRACTS

Veterinary Pathology: Laboratory animals

Veterinary Pathology: Others

SPEAKER ABSTRACTS

Resident Day | Wednesday 28th August 2024, 14.00-16.00

PICTURES OF PUBLICATION QUALITY, THE PERSPECTIVE OF THE PHOTO EDITORS AT THE VETERINARY PATHOLOGY JOURNAL

Silvia Ferro
University of Padua, Padova, Italy

Publishing accurate and high-quality images in a scientific journal is as crucial as publishing a solid research study, since images encapsulate and exhibit the outcomes of the research conducted. For this reason, an image must be realistic, perfectly centered on the lesion that should be easily identifiable, devoid of artifacts, and of the highest quality. The process begins with capturing the image. Photographing gross lesions requires taking precautions and using a special setup to ensure proper illumination. Similarly, capturing images through a microscope necessitates a thorough understanding of the instrument itself. Another vital component in producing a publishable image is the editing process. This presentation will explore these steps in detail, alongside the specific guidelines and standards set by the Veterinary Pathology Journal.
The significance of this topic stems from the pivotal role that images play in enhancing the comprehensibility and impact of scientific research. Images not only serve as proof of the findings but also facilitate a quicker understanding and appreciation of the complex details presented in the study. In the realm of veterinary pathology, where macroscopic and microscopic lesions provide critical insights into diseases, the ability to produce clear, informative, and accurate images is indispensable.

The first section of the presentation will focus on the technicalities of photographing gross lesions, emphasizing the importance of background selection, lighting, and camera settings that contribute to a well-composed and informative image. It will provide practical advice on avoiding common pitfalls and ensuring that the images accurately reflect the specimen's condition without introducing distortion or misleading details. Transitioning to microscopic photography, this segment will cover essential topics such as adjusting lighting, white balance, and focus to capture detailed images that are representative of the microscopic features observed. Special attention will be given to the use of the condenser to avoid chromatic aberration.

The editing phase is crucial in preparing an image for publication. However, it's imperative to maintain the integrity of the image, ensuring that the editing process enhances clarity without altering the scientific truth of the visual data. This section will discuss the ethical considerations in image editing, outlining what is considered acceptable in terms of cropping, adjusting brightness/contrast, and annotating images. We will also introduce the tools and software most suited for these tasks, providing attendees with the knowledge to select and utilize the appropriate resources for their image editing needs.

Finally, the presentation will review the Veterinary Pathology Journal's specific requirements and recommendations for submitting images. Understanding these guidelines is essential for researchers looking to contribute their findings to the scientific community through this esteemed publication. We will cover topics such as resolution standards, file formats, and preparing a plate of images.In conclusion, this presentation aims to equip veterinary pathologists and researchers with the knowledge and skills necessary to capture and edit images that not only meet the rigorous standards of scientific publication but also significantly contribute to the dissemination and understanding of their research findings. Through a combination of technical guidance, practical tips, and ethical considerations, attendees will leave better prepared to produce publication-worthy images that effectively communicate the essence of their work.

E-mail: silvia.ferro@unipd.it

SPEAKER ABSTRACTS

Resident Day | Wednesday 28th August 2024, 16.30-18.30

DEALING WITH RESIDENT'S CHALLENGES

Chiara Palmieri[1], Pompei Bolfa[2]
[1] *The University of Queensland, Australia*
[2] *Ross University, Saint Kitts And Nevis*

A supportive environment with balance between personal life and resident duties represents the key to a successful residency training program. Do you feel that you are part of such a program? Do you feel listened to when it comes to having an input in the veterinary pathology training in your institution? Do you feel valued? Are you efficient every day of your training? Are you a good communicator? Do you trust your resident mates and your senior pathologists? Our goal is to moderate a discussion in a safe space where we can listen to each other's ideas, concerns and experiences and get back with a better understanding of aligning expectations between different stakeholders. We will discuss chronic stress, burn-out, time-management, wellbeing and imposter syndrome, how to recognize them and ask for support if necessary. Residency is more than doing research, having to perform post-mortems, read slides, study and write reports.A happy and rested trainee is a productive trainee. By equipping residents with the necessary tools and resources, veterinary pathology training programs can cultivate competent and resilient pathologists prepared to contribute effectively to the field.

Pompei and Chiara have been residents before becoming senior pathologists and supervisors - they understand challenges, pressures and rewards of residency training. They hope that, drawing on their experiences and learning from your perspectives and insights, they can provide valuable guidance on how to navigate the complexities of veterinary pathology training and support all of you in achieving a healthy work-life balance.

E-mail: c.palmieri@uq.edu.au

SPEAKER ABSTRACTS

IATP Symposium | Wednesday 28th August 2024, 09.10-09.55

SCIENTIFIC EFFORTS FOR GAINING REGULATORY CONFIDENCE IN NAMS FOR NEUROTOXICITY

Ellen Fritsche
SCAHT - Swiss Centre for Applied Human Toxicology, Basel, Switzerland; 2 DNTOX GmbH, Düsseldorf, Germany,

Developmental (DNT) and adult neurotoxicity (ANT) represent critical areas of concern within neurotoxicology, focusing on the adverse effects of chemical exposures on the developing and adult nervous system, respectively. Addressing these concerns, the scientific community has made significant advances in deploying New Approach Methodologies (NAMs) for neurotoxicity assessment. NAMs promise a reduction in animal testing and an increase in throughput and human relevance of neurotoxicity testing.

A cornerstone in DNT evaluation has been the development of an in vitro battery (IVB) of assays. These assays are designed to capture adverse effects of compounds on a broad spectrum of neurodevelopmental processes, such as neural progenitor cell proliferation, migration neuron and glia differentiation, synaptogenesis and neural network formation. These neurodevelopmental key events are crucial for brain ontogenesis and hence provide ideal specimens of testing. Efforts are underway to likewise establish an integrated testing strategy for ANT within frameworks such as the Horizon Europe project PARC.

To enhance the applicability and confidence in these NAMs, biological applicability domains for both DNT and ANT are being defined. These domains outline the biological boundaries within which the NAMs can be considered predictive and include correlations with human or animal neuropathology and disease. Description of reliability and robustness complement the mechanistic validation of these assays. Currently, efforts are underway to reduce remaining biological uncertainties of the DNT IVB.

Collectively, these efforts signify a paradigm shift towards more humane, efficient, and predictive neurotoxicity evaluation aiming at safer chemical management and environmental health policies.

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SPEAKER ABSTRACTS

IATP Symposium | Wednesday 28th August 2024, 09.55-10.40

CURRENT STATE OF DEVELOPMENTAL NEUROTOXICITY (DNT) ASSESSMENT USING NEW APPROACH METHODOLOGIES (NAMS)

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In recent decades, there has been a notable increase in neurodevelopmental disorders among children, prompting concerns about the potential role of exposures to environmental chemicals. However, current in vivo testing methods often fall short in providing comprehensive developmental neurotoxicity (DNT) information on the amount of chemicals with limited available toxicity data. In response to this challenge, significant collaborative efforts have been undertaken by regulatory bodies, scientific communities, and stakeholders to develop an in vitro testing battery (IVB) to enhance DNT assessment. This battery focuses on key neurodevelopmental events, such as proliferation, migration, and differentiation, which if perturbed may lead to adverse neurological outcomes. Recently, the Organisation for Economic Co-operation and Development (OECD) released a crucial guidance document titled “Initial Recommendations on Evaluation of Data from the Developmental Neurotoxicity (DNT) In-Vitro Testing Battery” providing essential insights into the implementation and interpretation of the DNT IVB (OECD 2023). Moreover, integrated approaches to testing and assessment (IATA) case studies have been developed to exemplify diverse regulatory requirements including weight of evidence for hazard assessment, screening and prioritization (OECD 2022), and waiving of DNT in vivo studies. The DNT Health Effects Innovation (HEI) program at the National Institute of Environmental Health (NIEHS), is actively contributing to this endeavor by generating screening-level data on numerous compounds using the DNT IVB and developing IATA case studies to demonstrate its regulatory application.
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SPEAKER ABSTRACTS

IATP Symposium | Wednesday 28th August 2024, 11.10-11.50

LARVAL ZEBRAFISH AS AN ALTERNATIVE IN VIVO MODEL FOR ASSESSING SEIZURE LIABILITY: FROM BEHAVIOUR THROUGH TO FUNCTIONAL BRAIN IMAGING

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Seizures, and the related chronic disorder epilepsy, are complex neurological disorders for which animal models are critical to improve understanding and treatment. As well as occurring due to disease or head trauma, seizures can also occur as a side effect of new drugs, with 10%–20% failing to reach patients due to this 'liability' [1]. As current detection methods rely on time-consuming and costly electroencephalography (EEG) studies in rodents, many of these failures occur late in development. This means the negative animal welfare impact is twofold: firstly, rodent EEGs used to detect seizures are severe and invasive; and secondly many preclinical experiments using protected animals are undertaken unnecessarily on drugs that never reach patients. Importantly, seizures are electrographically consistent regardless of the underlying pathology and exhibit strong conservation across diverse taxa, including lower vertebrates [2]. As a result, over recent years the zebrafish has emerged as a credible alternative model in which to study seizures and epilepsy, showing conservation of basic brain structure and neurochemistry, as well as remarkably similar electrographic features compared with mammals. Importantly for early-stage safety assessment, the larval life stage (typically <7 days post fertilisation or dpf) offers higher throughput amenability, meaning it can be applied earlier in preclinical development than traditional rodent based approaches. The first studies employing larval zebrafish in seizure liability screens focussed on assessing convulsive behaviour as an indicator of seizure-potential [3]. However, these approaches only detect electrographic mechanisms resulting in altered behaviour and lack the sensitivity to differentiate between weakly proconvulsive, and non-seizurogenic, compounds.
To counter these limitations, we developed a functional imaging-based approach, using a non-protected life stage of a transgenic zebrafish (4dpf - pre independent feeding) that possesses a pan-neuronal genetically encoded fluorescent Ca^{2+} sensor, in which we can directly observe drug-induced seizurogenic activity [4]. When combined with light sheet fluorescence microscopy, this approach allows us to image a whole brain volume (circa 100k neurons) in around 1.5 seconds and affords an extremely powerful platform to assess the functional impact of neuroactive chemical exposure on the vertebrate brain. Assessment of model performance with compounds that activate a range of pharmacological targets associated with the induction of seizures in mammals has revealed mechanism-specific patterns of brain activity, and insights into anatomical regions that appear important for seizure initiation and progression [5]. Collectively these data suggest that this novel approach has the potential to reduce, or even replace, the use of mammals in new drug seizure liability assessment, and as a result minimise the number of drugs that carry this unwanted adverse side effect before they pass into the later stages of preclinical and clinical development.

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SPEAKER ABSTRACTS

IATP Symposium | Wednesday 28th August 2024, 11.50-12.30

ASSESSMENT OF CHRONIC NEUROTOXICITY USING NAMS

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Chemicals may trigger many types of neurotoxicity. Some are functional and reversible; others are associated with an irreversible loss of neurons. Especially severe and insidious is the loss of nigrostriatal dopaminergic neurons, the hallmark of Parkinson's disease and Parkinsonian syndromes. This is a small population of neurons for which damage is hard to discover by histopathological techniques, and functional deficits do not manifest before 50% of the neurons are lost. The capacity of chemicals to damage this neuronal subpopulation in humans is well documented: several poisonings with the chemical 1-methyl-4-phenyl-tetrahydropyridine (MPTP) occurred during the 1980s and have been found to result in severe parkinsonian motor deficits due to the loss of nigrostriatal dopaminergic neurons. The target of the bioactive MPTP metabolite 1-methyl-4-phenylpyridinium (MPP+) is the complex I (cI) of the mitochondrial electron transfer chain (ETC) and the pesticide rotenone has the same binding site on cI as MPP+. It has also been shown to cause nigrostriatal dopaminergic neuron loss associated with Parkinsonian motor deficits in rats. Epidemiological studies have shown a significant correlation between rotenone exposure and Parkinson's disease (PD). The findings with mitochondrial inhibitors in experimental models and in humans align with a well-accepted PD pathogenesis hypothesis that anchors on cI dysfunction. It may therefore be assumed that all chemicals that inhibit cI of dopaminergic nigrostriatal neurons have a propensity to cause parkinsonian motor deficits. EFSA started a project to provide a theoretical basis explaining the link between cI inhibition and neurotoxic adverse outcomes (manifesting in loss of dopamine neurons). This resulted in the development of AOP:3 (inhibition of cI leading to parkinsonian motor deficits) and its endorsement by the OECD. The AOP:3 thus became one of the best documented and one of the earliest fully endorsed AOP. It is a test/pilot case for exploring the use of AOP informed IATA in risk assessment. The basis of this approach is to use the AOP:3 to define a relevant point-of-departure (PoD) for risk assessment, to convert this PoD by an in vitro-to-in vivo extrapolation (IVIVE) to a threshold dose, and to use this to define acceptable daily intakes (ADI) or similar threshold measures. The AOP was initially exemplified by studies on rotenone and MPTP/MPP+. Subsequent work explored whether also other mitochondrial inhibitors might show similar effects and an extensive case study was performed on tebufenpyrad. This presentation will therefore introduce the AOP 3 and its implementation in the context of the EFSA project on Environmental Neurotoxicants.

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SPEAKER ABSTRACTS

Toxicological Pathology | Wednesday 28th August 2024, 14.15-14.55

SAMPLING THE NERVOUS SYSTEM FOR HISTOPATHOLOGY EVALUATION: GETTING ALIGNED TO DETECT NEUROTOXICITY

Deepa Bandi Rao

In toxicologic pathology, failing to examine appropriate tissues for specific test compounds raises the risk for false negative data upon which public health decisions are drawn by regulatory authorities. This is especially true for neuropathology where the expansive innervation into almost all other organ systems makes it impractical to screen each neural component for every routine regulatory toxicology study. Moreover, the neuroanatomical heterogeneity is intricately complexed within layers of neurophysiological and neurochemical frameworks that are also relevant for interpretation. Thus, the organization of the nervous system in context of function poses unique challenges in the evaluation of potential neurotoxicity. However, strategic sampling for histopathology in the context of study objectives remains a critical consideration for implementation in nonclinical toxicology study designs. This talk will provide an overview of the historical approaches in nervous system sampling, general best practices implemented in the past decade, and highlight some lessons learnt from implementation of recommendations with examples. Sampling approaches based on core concepts of neuropathology evaluation will be discussed, with a specific focus on integrated evaluation of relevant study design endpoints. Considerations for selective sampling to detect pathomorphological changes based on evolving knowledge from basic neurosciences will be included. This lecture will conclude with future directions in an effort to bridge existing gaps towards building strategic study designs for meaningful risk assessment.

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SPEAKER ABSTRACTS

Toxicological Pathology | Wednesday 28th August 2024, 14.55-15.20

BRAIN AND SPINAL CORD SAMPLING FOR MOLECULAR AND PROTEIN ANALYSIS

Xavier Palazzi
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Biodistribution studies in the context of ICH S12 (pharmacology and toxicology studies supporting early-phase clinical trials in the target population) require a specific methodology to sample fresh brain and spinal cord and allow for subsequent routine and investigative pathology, as well as molecular biology investigations. Extending the general concepts of central nervous system sampling to fresh brain sampling in cynomolgus monkeys, methods will be shared and discussed based on real life experience. Finally, an overview of the main regions of fresh brain sections will be provided to guide investigators.

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SPEAKER ABSTRACTS

Toxicological Pathology | Wednesday 28th August 2024, 15.20-15.45

DIRECT DRUG DELIVERY INTO BRAIN PARENCHYMA IN MONKEYS: TECHNICAL ASPECTS AND PATHOLOGY FINDINGS

Alexandra Duetting[1], Annette Romeike[1]
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Introduction
Neurological diseases are the most common cause of physical and cognitive disability worldwide and represent an emerging area of research. The exposure of the brain to systemically administered drugs is restricted by the blood-brain barrier. Hence, the development of new drug modalities, such as gene therapeutics, has recently underlined the importance of precise direct delivery.

Methods
71 cynomolgus monkeys were administered vehicle or capsid by intraparenchymal uni- or bilateral infusion into the thalamus or dentate nucleus. Application was performed by stereotactic surgery with frameless neuronavigation in general anesthesia. A post-surgical MRI was applied to verify correct targeting. After a defined observation period, animals were terminated and brains were examined macroscopically and microscopically.

Results
Surgery and recovery were without any reportable incidences in the majority of animals. In two animals dosing was only conducted unilaterally due to hemorrhages in the contralateral location. Findings in the MRI included typical post-surgical changes in normal ranges for most animals (hemorrhagic foci, air deposits, edema, fluid collection, and/or contrast enhancement). One animal exhibited neurological disorders. The post dose MRI revealed lethal bilateral bleeding at the injection sites. Procedure-related microscopic findings in scheduled necropsy animals were overall mild and included edema, gliosis, hemorrhages, pigment deposition, and local necrosis and/or nerve fiber degeneration.

Conclusion
Direct drug delivery into the brain of monkeys is a robust approach which can be used for various indications. The surgical procedure is overall well tolerated and complications are rare. Procedure-related microscopic findings are mild and can usually be clearly distinguished from any compound related effects.

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SPEAKER ABSTRACTS

Toxicological Pathology | Wednesday 28th August 2024, 15.45-15.55

SILVER STAINING IN NEUROPATHOLOGY AND NEUROTOXICITY: PRACTICAL APPROACHES, ADVANTAGES, AND DISADVANTAGES

Kristel Kegler[1], Klaus Weber[1]
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Silver stain has been mostly applied as a histochemical alternative in formalin-fixed, paraffin-embedded samples to identify normal neuroaxonal distribution/tract tracing or neuroaxonal degeneration within the central nervous system (CNS) in the context of neuropathology and neurotoxicity. From all protocols developed and improved up to date, modified Bielschowsky, modified methenamine-silver, Bodian, Gallyas (GAL) and Campbell–Switzer are the most commonly used in laboratory animals and in humans. From a practical point of view, the axonal affinity for silver depends not only on the protocol selected but also on the lesion to be evaluated. As examples, silver staining methods are selectively applied in detecting interrupted axonal tracts between CNS specific regions, in identifying senile plaques and neurofibrillary tangles present in aging or in Alzheimer's disease, in quantifying axonal damage and loss in multiple sclerosis, and for screening irreversible neuroaxonal injury caused by neurotoxic substances. Advantageously, silver stains can be combined with immunohistochemistry to colocalize altered functional or structural proteins within axons in an attempt to explain specific neuropathomechanisms. Hence, neuropathologists and neurotoxicologists should be aware of differences between silver methods and its targets, as well as the suitability of colocalization approaches to avoid ambiguities when interpreting results in animal models for CNS degenerative diseases or when assessing test-item induced neuroaxonal toxicity in pre-clinical studies.

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SPEAKER ABSTRACTS

Toxicological Pathology | Wednesday 28th August 2024, 15.55-16.05

IMMUNOHISTOCHEMISTRY CHARACTERIZATION OF GLIAL CELLS AROUND “HOLES” IN THE BRAIN - TWO SIMILAR EXPERIENCES IN THE DOG

Stefanie Arms[1], Enrico Vezzali[2]
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Glial cells in the central nervous system (CNS) are composed of macroglia, further divided in astrocytes, oligodendrocytes, and microglia. They make up more than half of the total cells of the CNS and are essential for neural functioning and repair. Any injury in the CNS is likely to be accompanied by a certain degree of gliosis, a nonspecific reactive change of glial cells. Depending on the cause of the insult, location and age of a lesion, a specific population or mixture of populations of glial cells will be present at the site. With few exceptions (e.g., presence of gitter cells/ gemistocytes) H&E stain may only serve to describe the morphology of a change. The use of specific antibodies for characterization of glial populations is immanent for the documentation of astrocytosis, oligodendrogliosis, or microgliosis which generally is preferred to Gliosis, not otherwise specified (NOS). Respective protocols are well established and accepted across many institutions. Here we present two similar vacuolar changes in the dog brain, where the characterization of glial cells with an array of immunohistochemistry markers was useful, facilitated considerations on pathogenesis, relevance, adversity, reversibility, or simply helped to better describe the morphologic changes. GFAP and SOX-9 for astrocytes, OLIG-2 and NG2 for oligodendrocytes, PDGFRα for OPCs - Oligodendrocyte Progenitor Cells, GSTπ for mature myelinating oligodendrocytes, and finally IBA-1 and CD68 for microglia will be discussed.

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SPEAKER ABSTRACTS

Toxicological Pathology | Wednesday 28th August 2024, 16.05-16.15

MYELIN METHODS

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Routine hematoxylin and eosin (H&E) staining is a suitable approach for detecting substantial structural changes in neural tissues but is less sensitive for detecting subtle findings to subcellular structures and various chemical constituents, including myelin. Special neurohistological methods are often used to better evaluate myelin integrity. Common options for light microscopy include acidophilic dyes (e.g., toluidine blue [used with hard plastic sections]); lipoprotein-binding dyes (e.g., Luxol fast blue [LFB], Weil's iron hematoxylin); lipid impregnation with metals (e.g., Marchi's, which uses osmium tetroxide for en bloc staining before embedding); and immunohistochemical (IHC) methods to highlight various myelin antigens (e.g., myelin basic protein [MBP] and peripheral myelin protein 22 [PMP22]). Some IHC methods reveal enhanced marker expression in damaged myelin (e.g., matrix metalloproteinase-9 [MMP9], S100). A myelin stain (typically LFB) is often used as one part of a two-component procedure to show two neural constituents simultaneously (e.g., to highlight myelin in relation to basement membranes, connective tissue, or Nissl substance in neurons). In neuropathology investigations, we use myelin stains as 2nd-tier methods (usually in combination with other neurohistological procedures to detect neuroaxonal injury and/or glial responses) to better characterize findings indicative of neural tissue injury if in-life neurological signs and/or microscopic findings in H&E-stained neural tissues suggest that nerve fibers generally or myelin specifically have been affected.

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SPEAKER ABSTRACTS

Toxicological Pathology | Wednesday 28th August 2024, 17.00-17.25

FINDING ONE'S WAY IN THE NERVOUS SYSTEM MAZE WITH AN AI COMPASS

Erio Barale-Thomas[1], Jogile Kuklyte[2], Laoise Bissett[2]
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This presentation will use material already discussed in the ESTP and other scientific meetings. However, we will concentrate on some pitfalls that we encountered, not for the CNS content (the Olney lesions being well characterised) but for the AI workflow. We intend to share our experience in this project and reiterate that optimal results need the collaboration of the pathologists' knowledge of the scientific question with the computational scientists' (CS) insight. We will also detail the too often undervalued expertise of the histology lab and reporting of study data (which we gather as pre/post-analytical steps).
Specifically in this case, optimal brain sampling (at necropsy) and histotechnique (for generating adequate sections), with the appropriate levels (Bolon, 2013) and good symmetry of both sides will guarantee an acceptable quality of the digital slides. Then due to some biological and technical variations, accurate AI tissue and level recognition will be required. The detection of the findings will heavily rely on the transfer of knowledge from the pathologists (the very subtle change of Olney lesions and the maze of brain anatomical regions) to the CS. The dataset size, due to the rarity of the findings, will demand interlaboratory collaboration in order to accurately train, test and validate the models. Manual assessment and reporting is time-consuming and repetitive; an automated process will surely help the pathologist in generating and communicating strong study results.
The maze that we refer to in our title is thus not only related to the brain anatomy but also to the complex tasks involved in evaluating the Olney lesions. Adding another complexity layer, we will explain the method's validation and generalisability, important steps towards the expansion and acceptability of digital pathology.

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SPEAKER ABSTRACTS

Joint Stream | Thursday 29th August 2024, 08.30-09.30

THE ROLE OF THE BRAIN BARRIERS IN CNS IMMUNITY DURING HEALTH AND NEUROINFLAMMATION

Britta Engelhardt

This lecture will cover our current understanding of the role of the endothelial, epithelial, glial and fibroblast brain barriers in maintaining CNS immune privilege. Based on our in vivo and in vitro observations I will address how alterations of barrier functions can impact on CNS immunity and contribute to neuropathology.

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SPEAKER ABSTRACTS

Toxicological Pathology | Thursday 29th August 2024, 11.00-11.45

PERFORMANCE OF BIOMARKERS NF-L, NSE, TAU AND GFAP IN BLOOD AND CEREBROSPINAL FLUID IN RAT FOR THE DETECTION OF NERVOUS SYSTEM INJURY

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Background
Target organ toxicity is often a reason for attritions in nonclinical and clinical drug development. Leveraging emerging safety biomarkers in nonclinical studies provides an opportunity to monitor such toxicities early and efficiently, potentially translating to early clinical trials. As a part of the European Union's Innovative Medicines Initiative (IMI), two projects have focused on evaluating safety biomarkers of nervous system (NS) toxicity: Translational Safety Biomarker Pipeline (TransBioLine) and Neurotoxicity De-Risking in Preclinical Drug Discovery (NeuroDeRisk).

Methods
Performance of fluid-based NS injury biomarker candidates neurofilament light chain (NF-L), glial fibrillary acidic protein (GFAP), neuron specific enolase (NSE) and total Tau in plasma and cerebrospinal fluid (CSF) was evaluated in 15 rat in vivo studies. Model nervous system toxicants as well as other compounds were used to evaluate sensitivity and specificity. Histopathologic assessments of nervous tissues and behavioral observations were conducted to detect and characterize NS injuries. Receiver operator characteristic (ROC) curves were generated to compare the relative performance of the biomarkers in their ability to detect NS injury.

Results
NF-L was the best performer in detecting both peripheral nervous system (PNS) and CNS injury in plasma, (AUC of 0.97 - 0.99; respectively). In CSF, Tau correlated the best with CNS (AUC 0.97), but not PNS injury. NSE and GFAP were suitable for monitoring CNS injury, but with lesser sensitivity. In summary, NF-L is a sensitive and specific biomarker in rats for detecting compound-induced central and peripheral NS injuries. While NF-L measurement alone cannot inform the site of the injury, addition of biomarkers like Tau and NSE and analysis in both blood and CSF can provide additional information about the origin of the NS injury.

Conclusions
These results demonstrate the utility of emerging safety biomarkers of drug-induced NS injury in rats and provide additional supporting evidence for biomarker translation across species and potential use in clinical settings to monitor drug-induced NS injury in patients.

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SPEAKER ABSTRACTS

Toxicological Pathology | Thursday 29th August 2024, 11.45-12.30

BEHAVIORAL TESTS IN DRUG SAFETY ASSESSMENT - ARE THEY RELEVANT FOR PATHOLOGISTS?

Andrea Greiter-Wilke
F. Hoffmann-La Roche, Basel, Switzerland

This presentation will give an introduction to non-clinical CNS testing mainly in rodents to assess compounds in development for adverse drug effects (Irwin screen, the locomotor assessment and the rotarod test). Case studies will complement the presentation, also highlighting the difference between general clinical observations and the more focussed CNS tests. Certain findings might result in a histopathological correlate, which will be an additional focus of the talk.

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SPEAKER ABSTRACTS

Toxicological Pathology | Thursday 29th August 2024, 14.15-15.00

NEUROPATHOLOGY THROUGH THE LENS OF TRANSLATIONAL IMAGING: OPPORTUNITIES FOR PRECLINICAL THERAPY ASSESSMENT

Nicolau Beckmann
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Imaging modalities like magnetic resonance imaging (MRI), nuclear tomographic imaging techniques, X-ray computed tomography and ultrasound are of paramount importance in the clinics for diagnostic purposes. In the past decades, imaging has also made inroads into drug discovery and development both at the preclinical and clinical levels.During this lecture, selected examples are going to be used to illustrate the usefulness of non-invasive imaging for preclinical pharmacological studies in small rodent models of diseases affecting the nervous system. Despite ongoing efforts to substitute animal experiments by in vitro or in silico methods, significant challenges arise in the study of complex regulatory processes of the cardiovascular, metabolic, respiratory or nervous systems, for instance, or in the investigation of pathology, especially when the disease mechanisms are poorly understood. Thus, animal experimentation remains central to examine disease as well as in the context of pharmacology, for example to validate potential drug targets, to assess therapeutic efficacy and to identify and validate biomarkers of compound efficacy and/or safety. The ability to image these small rodent models non-invasively opened the avenue to far-reaching applications in drug discovery and development (Rudin and Weissleder 2003; Beckmann et al 2004, 2007; Pien et al 2005). Distinct advantages of imaging to examine small rodent models are: (i) quantification of anatomical, functional, metabolic or molecular alterations within the animal's body with minimal distress. In particular, there is the possibility to generate information not accessible to ex vivo or post-mortem approaches regarding functional assessments; (ii) monitoring temporally and spatially animal models of diseases and the response to therapy. The pathology status pre- and post-administration of compound or vehicle can be easily compared in every single individual; (iii) repeated measurements allow each animal to serve as its own control, thereby benefitting statistical analyses and resulting in an estimated reduction of more than 80% in animal usage, depending on the application and the study protocol; (iv) adoption of imaging endpoints instead of time-consuming dissection and histology can significantly decrease the workload involved in tissue analysis and thereby speed up the evaluation of drug candidates; (v) potential to provide important information on the optimal timing and dosing of drugs. In particular, addressing therapeutic effects of compounds upon established pathology is feasible, with the option to stratify study groups based on imaging measures obtained just before treatment initiation; (vi) potential to detect and quantify liabilities of compounds (Hockings and Beckmann 2022; Obrecht et al 2023); (vii) the ability to go back and reanalyze images. Thus, when there are new biological questions/insights there is the option to avoid re-running animal experiments by first re-probing already acquired images; (viii) support of translational research: applying the same technique in animal models and in the clinic to study pharmacology enhances the confidence about the usefulness of a therapeutic agent. Moreover, back-translating learnings from the clinic contributes to refining the animal models and to enhancing the relevance of preclinical studies, since these can be guided by clinical requirements and questions as illustrated here through several examples regarding the central and the peripheral nervous system.

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SPEAKER ABSTRACTS

Toxicological Pathology | Thursday 29th August 2024, 15.00-15.45

ELECTROPHYSIOLOGY (EMG/NCV)

Veronika Stein
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Electrodiagnostics (Edx) are utilized to assess the functional integrity of the nervous system. They are essential for diagnosing patients with neuroanatomical lesions in the nerve root, peripheral nerve, neuromuscular junction, and muscle. Additionally, certain advanced electrophysiological tests can evaluate the central nervous system (such as motor evoked potentials, cord dorsum potentials, and electroencephalography). Edx offers critical insights into the distribution, severity, and nature of the underlying disease. However, to determine the cause of the disease, information from neurological examination, laboratory tests (including cerebrospinal fluid analysis), and muscle and nerve biopsies is necessary.
The electrodiagnostic tests most frequently used in clinical veterinary neurology are electromyography (EMG), peripheral motor (and sensory) nerve conduction velocity (NCV) measurement, and evaluation of the neuromuscular junction by supramaximal repetitive stimulation.

EMG represents the assessment of insertional, spontaneous, and voluntary electric activity of the muscle. As veterinary patients need to be anaesthetized due to difficulty of patient compliance in awake EMG, voluntary electrical activity is not routinely measured in veterinary medicine. The normal resting muscle (as in anesthesia) is electrically silent. However, there are four types of electrical activity seen in the normal muscle: insertional activity, miniature end-plate potentials (MEPPs), endplate spies, and motor unit action potentials (MUAPs).

There are also four patterns of abnormal spontaneous EMG activity: fibrillation potentials, positive sharp waves, complex repetitive discharges, and myotonic potentials. The thorough interpretation of these findings enables first insights into the distribution and severity of the disease. However, EMG has limited value in distinguishing between neuropathy and myopathy. Therefore, nerve conduction studies are mandatory to be added to the examination protocol.

NCV measurements can comprise evaluation of the motor and sensory component of peripheral nerves. For the technique, a recording electrode is placed in the muscle belly and the stimulating electrodes placed near the nerve examined. The current flowing between anode and cathode causes depolarization of the nerve and the propagation of the impulse along the nerve and via the neuromuscular junction to the muscle to create a compound muscle action potential (CMAP). Knowledge of the anatomic pathways allow many peripheral nerves to be evaluation with the radial and ulnar as well as the tibial and peroneal nerve being the most commonly examined nerves in the thoracic and pelvic limbs, respectively. For the evaluation of the motor component of the peripheral nerve, the motor nerve conduction velocity, the CMAP amplitude, duration and area need to be considered.

Despite the important information gained by these Edx techniques, the lack of an etiological diagnosis still necessitates biopsy and histopathological examination of muscle and nerve specimens by a specialized lab. With all information together a diagnosis is established and a therapy plan can be developed.

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SPEAKER ABSTRACTS

Toxicological Pathology | Thursday 29th August 2024, 16.15-17.00

MRI AND HISTHOPATHOLOGY IN MULTIPLE SCLEROSIS

Cristina Granziera

This lecture will focus on postmortem MRI, in combination with histopathology, performed in the brains of multiple sclerosis patients. We will focus on the challenges of postmortem MRI-histopathology experiments, on the importance of rigorous experimental settings as well as on the possibility of reaching ultra-high spatial resolution and cellular-molecular characterization in postmortem human tissue.

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SPEAKER ABSTRACTS

Toxicological Pathology | Thursday 29th August 2024, 17.00-17.45

CNS SAFETY BIOMARKER AND THEIR APPLICATION IN CLINICAL SETTINGS

Tobias Derfuss
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Laboratory and imaging biomarkers are meanwhile used routinely in clinical practice. They help to stratify patients to assign them to the right treatment, they help to monitor patients during treatment to ensure safety and treatment response and they are used to predict prognosis. This lecture will provide examples for the different aspects of biomarker use with a focus on neuroinflammatory diseases like multiple sclerosis and neuromyelitis spectrum disorders (NMOSD). The detection of autoantibodies against the antigens aquaporin 4 (AQP 4) and myelin oligodendrocyte glycoprotein (MOG) are examples for biomarkers that help to stratify patients to NMOSD. This has implications for treatment decision because certain treatments approved for MS might worsen NMOSD disease course. The antibody titre against the JC virus is a biomarker to assess the risk of individual patients to develop progressive multifocal leukoencephalopathy (PML). This is especially important before the start of the integrin α4β1 blocking antibody natalizumab that is associated with a high risk of progressive multifocal leukencephalopathy (caused by JCV). Another biomarker to assess the risk of PML in dimethylfumarate treatment is the lymphocyte count in peripheral blood. Due to the different modes of action this biomarker does not work for natalizumab. A novel biomarker that is currently introduced into clinical practice is neurofilament light chain. Neurofilament is a structural component of axons of the central and peripheral nervous system. When neurons are damaged it is released into blood and can be detected in increased concentration compared to healthy control cohorts. It can be used to monitor treatment and can also help to diagnose side effects like PML. Together with intrathecal IgM synthesis it is also used to predict the severity of MS at onset of disease. Several other biomarkers like intrathecal CXCL13, complement, and Chitinase-3-like protein 1 are correlated to the prognosis. Currently, trials are ongoing to evaluate prospectively the potential of biomarkers to guide treatment decisions in multiple sclerosis.

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SPEAKER ABSTRACTS

Veterinary Pathology | Thursday 29th August 2024, 11.00-13.00

VETERINARY DERMATOPATHOLOGY

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Clinical veterinary dermatopathology as a subspeciality requires the synthesis of clinical dermatology, histopathology and additional diagnostic tests to achieve a working diagnosis to advance patient care and outcomes. This fosters collaborative relationships between clinicians and pathologists. This interactive session with case-based discussion will cover a day in the life of a diagnostic dermatopathologist. Topics will range from infectious disease, immune-mediated entities and heritable disorders with a focus on companion animals. General approach to a skin biopsy sample and advisement of clinicians on lesion sampling will be covered. Common and uncommon diseases will be presented including histopathologic findings, a discussion of differential diagnoses and correlation with clinical lesions.

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SPEAKER ABSTRACTS

Veterinary Pathology | Thursday 29th August 2024, 11.00-12.00

TB IN CAPTIVE AND FREE-RANGING WILD ANIMALS

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Tuberculosis (TB) is a worldwide present chronic bacterial infection caused by members of the Mycobacterium tuberculosis complex (MTC) including M. tuberculosis, the main causative agent of human TB, and M. bovis, the main one for (zoonotic) animal TB. Both M. tuberculosis, M. bovis, and other members of the MTC do infect wild animals, with broad implications for public health, animal farming, and conservation. Members of the MTC can survive for some time in the environment and have the capacity to infect multiple host species. These characteristics explain TB epidemiology and represent barriers to TB control (Gortázar et al. 2023). Tuberculosis in wild animals occurs in a broad range of settings, from zoos and other captive animals, through fenced populations, over to completely free-ranging wildlife. This presentation addresses the relevance of TB in different settings and uses case-studies to understand the complexity of TB epidemiology at the interface with livestock.

Host communities
Wildlife is seldom alone: rather than accepting the classical single or two-host system view where only certain species were regarded as maintenance hosts, we now try to understand MTC dynamics in complex multi-host MTC maintenance communities where several different wild and domestic host species and the environment contribute to build a network that facilitates pathogen survival and spread (Barroso and Gortázar 2024). An ongoing study of MTC host communities of the Iberian Peninsula demonstrates that host community characteristics drive the risk of tuberculosis for wildlife and for livestock. High TB prevalence host communities are characterized by a high relative weight of wild ungulates. That is, communities dominated by overabundant wild ungulates are those at greatest risk.

Wildlife-derived new tools
Research on wildlife hosts has provided benefits for the global TB research community and for TB control in livestock, including new diagnostic tools such as eDNA sampling, a new vaccine, and novel strategies for integrated TB control at the interface. Sponge-based eDNA sampling is an extremely useful tool that allows investigating the circulation of MTC in a non-invasive way, both on animals and in environmental samples such as latrines, dens, pastures, or watering and feeding sites. Applications range from epidemiological risk analysis (Martínez-Guijosa et al. 2020) to farm biosafety measure assessment and on-farm pathogen monitoring (Herrero-garcía et al. 2024). Starting from the wild boar model, Garrido et al. (2011) developed an immunostimulant based on heat-inactivated M. bovis (HIMB) which has demonstrated to reduce the mycobacterial load in target tissues and tuberculous lesions in several wild and domestic animal species. Furthermore, immunization with HIMB has shown cross-protective capacity against unrelated pathogens such as Leishmania in mice (Ferreras-Colino et al. 2023) and Salmonella in swine (Vaz-Rodrigues et al. 2022). This has opened new avenues for vaccine research applicable to a broad range of pathogens, host species, and settings (Juste et al. 2022). Other interventions against animal TB benefiting from wildlife research include significant recent innovations in farm biosecurity assessment (Martínez-Guijosa et al. 2021). In summary, results generated on wild animals produce epidemiological insights, identify intervention opportunities, and contribute to developing new tools for TB control.

SPEAKER ABSTRACTS

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SPEAKER ABSTRACTS

Veterinary Pathology | Thursday 29th August 2024, 14.15-15.00

INVERTEBRATE PATHOLOGY: APPROACH TO DIAGNOSTICS AND COMMON DISEASES, INCLUDING INFLAMMATION, INFECTION, ENVIRONMENTAL STRESS/TOXIN EXPOSURE, NEGATIVE ENERGY BALANCE, AND NEOPLASIA

Elise LaDouceur[1, 2]
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Invertebrates serve as keystone species in their ecosystems (e.g., corals, bees, sea stars), charismatic mascots in zoos and aquaria (e.g., octopuses), and beloved pets (e.g., tarantulas, hermit crabs). Invertebrate pathology is a growing discipline that benefits greatly from the unique insight and comparative knowledge of veterinary pathologists. Veterinary diagnosticians are often hesitant to engage in this field as residency training programs typically provide limited exposure to invertebrate species. One of the primary pathology journals, Journal of Comparative Pathology, still excludes invertebrate manuscripts. Fortunately, other journals and textbooks now recognize the value that veterinary diagnosticians add to invertebrate pathology. Veterinary Pathology, the official publication of the American College of Veterinary Pathology, recently dedicated an entire issue to invertebrate diseases, and the textbook “Pathology of Wildlife and Zoo Animals,” has an invertebrate chapter. A primary hurdle in reviewing invertebrate cases is understanding basic histologic responses to injury. In addition to the above references, the textbook “Invertebrate Pathology” is a comprehensive reference featuring descriptions of invertebrate defense mechanisms, as well as succinct descriptions of main diseases across invertebrate taxa. The most commonly encountered histologic lesion is inflammation. Inflammation in Arthropoda, arguably the largest invertebrate taxon, results in infiltration of hemocytes (the primary immune effector cell) and/or melanization. Hemocyte is the general term for immune cells across most invertebrate taxa with the exception of cnidarians, whose immune cells are termed amoebocytes, and echinoderms, whose immune cells are terms coelomocytes. Inflammation can be the result of sterile processes, such as toxins and trauma, but is often associated with infection. Although veterinary pathologists will be familiar with those infectious agents that can infect both vertebrates and invertebrates, there are numerous infections unique to invertebrates, requiring diagnosticians to delve into the literature, flex their comparative skills, and employ ancillary diagnostics (e.g., electron microscopy, PCR, culture, etc.). Viral infections may cause unfamiliar inclusions, such as those of nucleopolyhedrosis viruses, which lack a vertebrate counterpart. Hyphal diseases in invertebrates are numerous, many of which have life stages that are uncommonly or never observed in vertebrate tissues (e.g., protoplasts and hyphal bodies), or mechanisms for immune-system evasion making distinction of postmortem versus antemortem disease particularly challenging (e.g., entomopathogenic fungal infections). Protozoa that cause classic diseases in invertebrates, such as perkinsosis in abalone, are only recently described in vertebrate species (e.g., systemic Perkinsus infection in frogs). Some metazoan parasites (e.g., cephalopod renal dicyemids) have no known vertebrate counterpart. Other diseases that are routinely identified histologically in invertebrates include environmental stress/toxin exposure, negative energy balance, and neoplasia. Invertebrates are exquisitely sensitive to environmental perturbations. Relative minor changes in water quality parameters and low levels of toxins that may be well tolerated in vertebrates can cause mass mortalities in invertebrates. Corals are a classic example, where minor changes in pH, sedimentation, temperature, etc. can lead to widespread zooxanthellae degeneration (“bleaching”) and epithelial necrosis. Negative energy balance, which is evident histologically in vertebrates as adipose atrophy, can be challenging to diagnose in invertebrates, who lack adipose tissue. Few studies have published results of controlled experiments on histologic lesions of negative energy balance in invertebrates. Horseshoe crabs that are starved have histologic reduction in cytoplasmic globules of the hepatopancreas. Similarly, cephalopods that are anorexic during senescence consistently have decreased cytoplasmic globules in the digestive gland, which is analogous to the hepatopancreas and is the energy storing organ of cephalopods. Using comparative histology and knowledge of functional anatomy, veterinary diagnosticians can make inferences into the general energy status of an animal, and in turn provide useful information to clinicians and biologists managing these species. Neoplasia is challenging to diagnose in invertebrates because it requires a broad understanding of tissue responses to injury in order to discern neoplasia from a reactive or inflammatory lesion. This is particularly true for hemocytic neoplasms, which can be distinguished from inflammatory lesions in arthropods by the lack of melanization, lack of capsule formation, and broad sheets of cells. This lecture will cover useful tips to approaching invertebrate cases, including key references. Common diseases encountered in invertebrates will be discussed, including inflammation, infection, environmental stress/toxin exposure, negative energy balance, and neoplasia. Hopefully, joy will spark and even the most trepidatious diagnosticians will be inspired to delve into invertebrate pathology.

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SPEAKER ABSTRACTS

Veterinary Pathology | Thursday 29th August 2024, 15.00-15.45

OVERVIEW OF TRANSMISSIBLE CANCERS IN BIVALVE MOLLUSCS

Antonio Villalba
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Cancers in bivalve molluscs
Bivalve molluscs provide multiple ecological services; some species are key factors for the health of littoral ecosystems (Smaal et al., 2019). Fishery and culture of bivalve molluscs have very high socioeconomic relevance for many coastal human communities. Therefore, diseases of bivalve molluscs are matter of concern, particularly those causing mass mortalities. Bivalve molluscs, as any other animal group, can be affected by cancers and multiple types of tumours affecting bivalve molluscs have been described. Among them, most research effort have been focused on those reaching epidemic levels due to their devastating potential and their economic and ecological consequences. Two types of cancers reach epidemic levels in bivalve populations, the so called disseminated neoplasia and gonadal neoplasia.

Disseminated neoplasia
The name “disseminated neoplasia” (DN) is applied to a disease characterised by the uncontrolled proliferation of abnormal (cancerous) cells through the connective tissue and vessels in multiple organs. It has been reported as “leukaemia” in some bivalve species but the less compromised denomination “disseminated neoplasia” became more frequent because of the lack of unequivocal demonstration of haemopoietic origin of the proliferating cells. Likely, various types of cancer are grouped under DN. Since late 1960s, high (even › 50%) prevalences of DN have been reported in mussels, clams, oysters and cockles from different continents, frequently associated with mass mortalities (Carballal et al., 2015).

Disseminated neoplasia aetiology
An obvious question arose soon: what is the cause of these neoplastic disorders reaching so high prevalence? Multiple causes were proposed but two hypotheses were considered as the most probable, pollution and infectious agents. Eventually, experimental transmission to healthy individuals was achieved by co-habitation in tanks with DN-affected individuals and by injection of haemolymph withdrawn from DN-affected individuals. Thus, the infectious aetiology became more widely accepted and the search for infectious agents was addressed. The involvement of RNA viruses was suspected. The answer to this long-lasting enigma was provided by Metzger et al. (2015), who demonstrated that DN is spreading among softshell clams, Mya arenaria, through the east coast of North America as a clonal horizontally transmissible cell derived from a single original clam. In other words, no infectious agent is involved in transmission but the own cancer cells that are released to the seawater from a DN-affected clam can be taken from the water by a healthy clam and, eventually, some of those inhaled (through the clam syphon) cancer cells can penetrate and proliferate through the recipient-clam tissues, and so on. Soon later, the horizontal transmission of a cancer cell clone was also demonstrated for the DN of the northern blue mussel, Mytilus trossulus, in Canada; the occurrence of two different cancer cell clones being horizontally transmitted among common cockles in Galicia (NW Spain) was shown and, surprisingly, the cancer cells spreading among golden carpet shell clams, Polititapes aureus, in Galicia, were demonstrated to derive from another clam species, Venerupis corrugata (Metzger et al., 2016). Since then, more cases of intra- and interspecies transmission of cancer cells has been demonstrated explaining the spread of DN in different bivalve species. Cancer cells released from DN-affected individuals can survive for long periods, even various weeks, in seawater, which favours DN spreading (Giersch et al., 2022).

SPEAKER ABSTRACTS

Gonadal neoplasia

Gonadal neoplasia (GN) results from the transformation and uncontrolled proliferation of the germ cells of gonad follicles, which can fill the follicle lumen and escape into the connective tissue and invade other organs. This disease has been reported from a number of bivalve mollusc species. Nevertheless, high prevalences of GN have been detected only in few species. The mortality associated with GN is lower than in the case of DN. Likely because of this, much less research effort has been devoted to GN. Its aetiology is unknown and transmissibility has not been proved (Carballal et al., 2015).

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SPEAKER ABSTRACTS

Veterinary Pathology | Thursday 29th August 2024, 16.15-17.00

TEACHING VETERINARY PATHOLOGY TO VETERINARY MEDICINE STUDENTS: WHAT EUROPEAN PERSPECTIVE?

Massimo Castagnaro
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The COVID-19 pandemic and its consequences on teaching methodologies and activities have provided an unexpected opportunity to reconsider what constitutes essential, relevant or unnecessary knowledge and skills in Veterinary Pathology (VP), redefining the Intended Learning Outcomes (ILOs) for the undergraduate students in the European Veterinary Medicine (VM) programmes.
VP is a large and complex area of knowledge (Kn), skills (Sk) and competences (Co) encompassing the teaching of a wide range of subjects including General Pathology, General and Special Anatomical Pathology, Pathophysiology, Post-Mortem Techniques, Molecular Pathology, as well as several other specialized fields such as Ultrastructural Pathology, Histopathology, Cytopathology, and Organ Pathology (Neuropathology, Nefropathology, Dermatopathology...) among others. Furthermore, in many European Countries, some of these subjects are regularly taught in several Veterinary Science-related programmes such as Veterinary Assistant/Nurse and Animal Production. The focus of the lecture will be on VM programmes, where the goal of teaching VP is to allow students to reach a level of ILOs (Kn, Sk and Co) adequate to the needs of the veterinary profession.
Setting realistic and appropriate ILOs in all pertinent subjects for VM undergraduates is critical and requires a clear understanding and definition of Kn (content of technical information), Sk (abilities/techniques) and Co (applied Kn and Sk in a variety of professional contexts) across relevant subjects. Valuable guidance in this endeavor is provided by the Standard Operating Procedures (SOPs) of the European Association of Establishment for Veterinary Education (EAEVE)[1] that embodies Kn, Sk and Co within the framework of the so-called Day-One Competences (DOCs). The latter categorize VP into two main areas: General Pathology (GP) and Diagnostic Pathology (DP). While DP has a direct professional impact in several Clinical and Public Health settings, GP is at the base for establishing and advancing Kn across all professional subjects and therefore requires a continuous discussion with colleagues responsible of all GP-related subjects. The methods to assess the degree of achievement of ILOs in each student should coherently stem from their adequate definition. In addition to an adequate definition of ILOs, another important critical factor in the teaching of VP has been identified by the Standards and Guidelines for Quality Assurance in the European Higher Education Area (ESG, 2015)[2] where in section 1.3 it is stated that “Institutions should ensure that the programmes are delivered in a way that encourages students to take active role in creating the learning process, and that the assessment of students reflects this approach”. As a matter of fact, it is an integral aspect of academic teaching experience to acknowledge the continuous and rapid evolution of cognitive functions and specific learning abilities along different student cohorts[3]. These critical factors collectively pose significant challenges to the efficacy and efficiency of teaching VP. This interactive lecture aims to address and discuss with the participants these challenges, to share and compare the efficacy and pinpoint specific hurdles of different European teaching systems, and to exchange suggestions and consideration about future perspectives. Additionally, the lecture will explore the use of innovative teaching methodologies that can enhance the teaching and learning experience in VP. The lecture will be carried out as a progressive, step-by-step set of points aiming to reach, for each one of them, a common draft position paper.

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SPEAKER ABSTRACTS

Joint Stream | Friday 30th August 2024, 08.30-09.30

A FANDANGO OF FORM AND FUNCTION: THE INTERPLAY BETWEEN CLINICAL NEUROLOGY AND NEUROPATHOLOGY INVESTIGATION

Brad Bolon
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The central (CNS) and peripheral (PNS) nervous systems of vertebrate animals are arbitrary divisions of a continuous, body-wide communication grid based on shared principles of neuroanatomic organization, albeit with many phylum- and species-specific adaptations. Discrete anatomic domains are associated with specific neural activities, so neural dysfunction observed clinically affords a useful guide to optimize neural sampling and neuropathology evaluation. Neurological syndromes are differentiated by patterns of clinical abnormalities that provide guidance regarding affected locations, and sometimes the causes, of neural diseases. A particular constellation of neurological signs related to damage affecting a given nervous system structure develops regardless of the inciting cause. Lesion localization is influenced by both intrinsic tissue properties (e.g., high metabolic rates and nutrient requirements of neurons) and extrinsic factors (e.g., migration of cells or pathogens in blood vessels and/or the fluids flowing through or around neural tissues). Adaptation of neural sampling and analytical protocols to target anatomic sites correlated to the specific signature of neural dysfunction provides a rational way to improve the likelihood that a neuropathology evaluation will discover the lesions and etiologies that are responsible for the spectrum of in-life neurological signs in both the diagnostic and experimental pathology settings.

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SPEAKER ABSTRACTS

Toxicological Pathology | Friday 30th August 2024, 09.30-10.00

BRIDGING THE GAP: UNDERSTANDING DEMENTIA THROUGH ANIMAL MODELS

Laura Fusaro
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Dementia is not a specific disease but rather a complex group of symptoms and conditions associated with decreased memory, thinking skills, reasoning, communication, and the ability to perform everyday activities. The pathogenesis of dementia involves a combination of genetic, environmental, and age-related factors and depends on the specific type of dementia, but common underlying mechanisms and critical pathological hallmarks are associated with the disease, such as neurodegeneration, protein misfolding and aggregation, and neuroinflammation. Animal models have played a pivotal role in unraveling the pathophysiological mechanisms underlying this condition, offering invaluable insights into understanding the molecular pathways implicated in disease pathogenesis, identifying biomarkers, and testing therapeutic strategies before human clinical trials. However, it is essential to recognize the significant challenges they pose, including species differences in brain anatomy and physiology, the inability to recapitulate the complexity of human dementia fully, and challenges in modeling disease heterogeneity. This presentation provides an overview of the animal models utilized in dementia research, focusing on Alzheimer's disease and frontotemporal dementia. It reviews not only the pathology from well-established models, including transgenic mice and non-human primates, highlighting their strengths, limitations, and translational relevance but also provides an overview of the recent advancements in the ever-evolving and challenging therapeutic landscape.

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SPEAKER ABSTRACTS

Toxicological Pathology | Friday 30th August 2024, 10.00-10.30

AUTOIMMUNE NEURODEGENERATIVE DISEASES: SHIFTING PARADIGMS AND EMERGING OPPORTUNITIES

Dinesh Bangari
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Neurodegenerative diseases, including autoimmune variants, burden global healthcare. Autoimmune neurodegenerative diseases such as multiple sclerosis, Guillain-Barré syndrome, and neuromyelitis optica spectrum disorder result from a breach in immune self-tolerance to neural proteins. With heterogeneous clinical presentation and characteristic lesions typified by chronic inflammation and progressive tissue damage centered on myelin, these disorders remain elusive in terms of underlying pathophysiology and effective disease-modifying therapies. However, recent explorations have unraveled novel and druggable pathomechanisms. Novel therapeutic targets and treatment approaches thus emerge, which necessitate rigorous evaluation in nonclinical in-vitro and in-vivo models. In this regard, nonclinical pathology and toxicology teams, endowed with cross-disciplinary subject matter expertise and supported by molecular pathology (omics) toolkits and machine-learning augmented workflows, are poised to play a critical role in the discovery and development of efficacious and safe medicines for autoimmune neurologic disorders. This presentation will elucidate/illustrate the role of translational models in autoimmune neurodegenerative disease drug discovery and offer pertinent perspectives for nonclinical therapeutic efficacy and safety testing.

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SPEAKER ABSTRACTS

Toxicological Pathology | Friday 30th August 2024, 11.00-11.45

USING ADVANCED HUMAN IN VITRO MODELS TO STUDY NEURODEGENERATIVE DISEASE

Jeroen Pasterkamp
University Medical Center Utrecht, Utrecht, Netherlands

The goal of our work is directed towards understanding how neural circuits form during development and why they change or degenerate during disease. For this research, we use a combination of molecular cell biological approaches (e.g. scRNAseq, CRISPR), (3D) microscopy, mouse genetics and iPSC-based modelling in combination with microfluidics. Here I will focus on our work that attempts to dissect the molecular mechanisms underlying neurodegenerative diseases, in particular amyotrophic lateral sclerosis (ALS). ALS is a fatal neurodegenerative disorder with a lifetime risk of 1:400, affecting upper and lower motor neurons. Loss of motor nerves leads to weakness of skeletal muscles, ultimately resulting in death 3-5 years after diagnosis. Treatment options for ALS are limited and the development of new therapeutic strategies requires further insight into the pathogenic mechanisms underlying ALS. In addition to employing ALS animal models, we have invested in setting up a wide array of advanced in vitro systems generated from human induced pluripotent stem cells (iPSCs) in combination with sensitive readouts. These models range from individual cell types, such as motor neurons or skeletal muscle cells, to combinations of cell types in microfluidic devices and even engineered 3D tissues (organoids). We have developed several neural organoid protocols for analyzing 3D neural tissue and specific cell-cell interactions. Importantly, these models show established pathological hallmarks of ALS as well as pathogenic changes and can therefore be used to further dissect disease mechanisms and to identify therapeutic targets.

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SPEAKER ABSTRACTS

Toxicological Pathology | Friday 30th August 2024, 11.45-12.30

UPDATE ON CANNABINOID-BASED THERAPEUTIC OPTIONS FOR TREATING NEURODEGENERATIVE DISORDERS

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Cannabinoids form a singular family of plant-derived natural compounds and synthetic derivatives able to exert multiple biological actions in the human body, which derive from their ability to mimic, block or modulate the action of various endogenous signalling lipids that form part of the so-called endogenous cannabinoid system. Many of these actions have been found to have potential therapeutic applications in human pathologies. The regulation of cell homeostasis, integrity and survival in different tissues but, in particular, in neural cells, is one of the different biological actions of endocannabinoids that is attracting more interest, as it explains why the pharmacological modulation of different elements of the endocannabinoid system may afford benefits in pathologies related to brain damage, in particular in chronic progressive neurodegenerative disorders. These beneficial effects appear to be facilitated by the location of those endocannabinoid elements modulated by plant-derived or synthetic cannabinoids (e.g., CB_1, CB_2, GPR55, PPAR-γ receptors, endocannabinoid inactivating enzymes) in cellular substrates (e.g., neurons, astrocytes, microglial cells, neural progenitor cells, blood-brain barrier) that are important in the control of neural cell survival. This fact allows these endogenous compounds to be active in the preservation, rescue, repair and/or replacement of neurons and other neural cells against the numerous insults that contribute to potentially damage these cells in neurodegenerative disorders. This lecture will attempt to update the most recent and relevant experimental evidence, mainly obtained at the preclinical level, supporting that different endogenous, plant-derived or synthetic cannabinoids may behave as neuroprotective and neurorepair agents in Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS), Huntington's disease, spinocerebellar ataxias, and also in accidental brain damage (e.g., stroke, brain trauma, spinal injury). Examples of this preclinical development in ALS will be presented in the lecture, included the work conducted in the canine form of ALS so-called degenerative myelopathy. The major conclusion will be that the experimental evidence collected so far has already reached the necessary biomedical relevance to move towards a prompt clinical validation of cannabinoid-based medicines for these disorders. In this sense, cannabinoid-based clinical trials have been already initiated in some of these disorders or will be initiated soon in others as ALS using, for example, cannabinoids that selectively activate the CB_2 receptor.

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SPEAKER ABSTRACTS

Toxicological Pathology | Friday 30th August 2024, 14.15-14.35

PAST CURRENT AND FUTURE OF DEVELOPMENTAL NEUROTOXICITY STUDIES

Frédéric Schorsch
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The concern that industrial chemicals, agrochemicals or pharmaceuticals can contribute to the increase in neurodevelopmental disorders is not new. The developmental neurotoxicity (DNT) studies in rodents (OECD Test Guideline 426, OECD, 2007) have been proposed already some decades ago to detect chemicals having clear effects on brain development.

The TG 443 (extended one-generation reproductive toxicity study) (OECD, 2018) for chemicals, and more recently the “Nonclinical Safety Testing in Support of Development of Pediatric Medicines” S11 guidance document for pharmaceuticals have extended more broadly the scope of the investigation of chemicals on the brain development.

Despite this progress there is still a lot of remaining scientific gaps: if a strong decrease of thyroid hormones is known to affect the brain development, the role of thyroid toxicants to adverse neurological development according to their potency or their mode of action (direct or indirect via thyroid hormone catabolism) is currently under scrutiny.

This lecture will review the contribution of DNT studies in our knowledge about neurodevelopmental toxicants and how they are currently used. In a second part the limitations of DNT studies will be discussed and we will present some New Approach Methodologies like the DNT in vitro testing battery (IVB) to derisk a higher number of chemicals. We will show how they take more and more importance in the regulatory risk assessment of pesticides.

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SPEAKER ABSTRACTS

Toxicological Pathology | Friday 30th August 2024, 14.35-15.00

DEVELOPMENTAL NEUROPATHOLOGY: INTRODUCTION AND OVERVIEW

Wolfgang Kaufmann
Independent Consultant Toxicologic Pathology, Bad Duerkheim, Germany

The presentation will include introductory remarks and will focus on the neuropathology component of the DNT-rat animal model. Neuromorphological examinations are performed at two time points, as a rule PND 21 and PND 62 - 70, as a minimum. The examination at two time points at the end of dam/ pup treatment (PND 21) and at the adolescent stage of the offspring (PND 62 - 70) is crucial to detect true developmental effects and to decide, whether an effect is transient or persistent. Neuropathology performed during neurodevelopment faces the challenge to differentiate between normal, physiological changes due to the growth process from a growth retardation or maldevelopment, which may be very discrete or dramatically obvious. Single developmental neuropathological outcomes are presented as examples, in particular MAM, an antimitotic compound given to dams. Typical neurodevelopmental lesions found in the brain are neuronal heterotypias indicating a wrong neuronal settling and a neuronal cell hypoplasia in hippocampus and cortex cerebri regions. No neurodevelopmental alterations were found in the cerebellum. A reasoning for the specific impact of MAM treatment on the development of the hippocampus and cerebral cortex is given. The presentation will finally give attention on the interpretation of neuropathology data collected in a DNT-study and the necessity to evaluate them in an overall context of the study and available information of the tested compound in a “weight-of-evidence” approach.
Abb.: DNT = Developmental Neurotoxicity, PND = postnatal day, MAM = Methylazoxymethanol

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SPEAKER ABSTRACTS

Toxicological Pathology | Friday 30th August 2024, 15.00-15.45

MORPHOMETRICS IN DEVELOPMENTAL NEUROTOXICITY STUDIES- PITFALLS AND INTERPRETATION

Heike Marxfeld
BASF SE, Frankenthal, Germany

The morphometric measurements of histological sections of the brain as required by OECD guidelines for in vivo developmental neurotoxicity studies (OECD 443 and 426) will be discussed. The workflow to obtain these measurements will be critically reviewed and potential biases explained. From necropsy and perfusion fixation to embedding and sectioning in the correct orientation there are potential pitfalls which will be discussed. Historical control data for these measurements will be reviewed in context with other parameters like brain and terminal body weight as well as length and width measurements of the brain. Suggestions for interpretation of these measurements will be given and discussed with the audience.

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SPEAKER ABSTRACTS

Toxicological Pathology | Friday 30th August 2024, 16.15-16.45

THYROID HORMONE IMBALANCES - TOX ASPECTS

Stephanie Melching-Kollmuss

Substances inducing thyroid histopathological changes and/or thyroid hormone changes in rodents are under specific focus of European Regulatory Assessments since criteria for endocrine disruption are in place. Following the European Directives 605/2018 and 2100/2017, a plant protection active ingredient or a biocide is to be considered an endocrine disruptor, if it causes and adverse effect, it shows endocrine activity, and there is evidence for a plausible link between the adverse effect and the endocrine activity.

Thyroid hormone imbalance and / the observation of thyroid histopathological effects in rodents is considered to be of concern with regard to the adverse outcome neurodevelopmental toxicity and further studies and assessments are required following the ECHA / EFSA Guidance Document, 2018.

The ECETOC Thyroid Task Force has worked over the last 5 years to establish a science based testing strategy to identify maternal thyroid hormone imbalance and neurodevelopmental effect in the progeny, and has published a series of 4 papers. Within this investigation also a large dataset of rat studies with data on thyroid function, brain and/or further neurodevelopmental parameters. As brain-related parameters late-stage key events (e.g. alterations in electrophysiology or auditory signaling), neurobehavioural effects on the organism level (motor activity, acoustic startle response, learning and memory), structural changes in brain and expression of brain genes were assessed. The rat case studies were sorted along thyroid modes of actions, e.g. TPO (thyroid peroxidase) inhibition, NIS (sodium iodide symporter inhibition / iodine deficiency, and increased thyroid hormone clearance (liver enzyme inducers and substances interacting with thyroid hormone transport). Correlations between thyroid hormone imbalance in offspring and the occurrence of neurodevelopmental toxicity have been made.

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SPEAKER ABSTRACTS

Toxicological Pathology | Friday 30th August 2024, 16.45-17.15

THYROID HORMONE IMBALANCE IN PREGNANT RATS AND ITS IMPACT ON NEURODEVELOPMENT IN THE PUPS : A HUNT FOR A RELIABLE HISTOPATHOLOGICAL BIOMARKER

Babunilayam Gangadharan

It has been known for a long time that thyroid hormone imbalance in pregnant mothers will have deleterious effect on the neurodevelopment of the child.
Since 2018, market authorization of a pesticide or a biocide in Europe requires hazard assessment of endocrine disrupting properties includeing the Thyroid modality (EC, 2018). Precisely, substances inducing thyroid histopathological and/or thyroid hormone effect in rodent studies need to be further investigated to rule out whether the substance can be considered as an endocrine disruptor for the thyroid pathway, including possible neurodevelopment impact in the pups.

Recently, Thyroid Task Force of European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) and Crop Life Europe (CLE) has proposed a Tiered Tesing and Assessment Scheme (Thyroid- NDT-TAS) to identify thyroid hormone disruptors and neurodevelopmental effects in the progeny (Melching Kolluss et al 2023). Histopathological assessment of the brain to identify a reliable biomarker to assess the neurodevelopmental effect is an important aspect in this testing sheme. Periventricular heterotopia in the corpus callosum and pesistence of external granular layer in the cerebellum are being proposed as potential histopathological biomarkers (Goodman and Gilber, 2007; Minami et al 2023). Histopathological correlate in the cochlea for the hearing impairment observed in the rat pups derived from hypothyroid dams is another potential biomarker. This presentation will give a brief overview on this aspects including some unpublished observations from our laboratory.

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SPEAKER ABSTRACTS

Veterinary Pathology | Friday 30th August 2024, 11.00-12.00

TUMOURS OF THE UROGENITAL SYSTEM: THE OLD, THE NEW AND THE FUGITIVE!

Chiara Palmieri[2], Valeria Grieco[1]
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Over time, our understanding of the tumours affecting the intricate urogenital system has evolved, with new discoveries shedding light on previously unrecognised entities, redefining traditional classification and updating grading systems. This presentation aims to comprehensively explore the spectrum of tumour within the urogenital tract, encompassing the emergence of novel entities (“the new”), the re-evaluation of historical perspectives (“the old and the fugitive”) and the integration of updated knowledge into veterinary pathology practice. Recent advancements have unveiled new tumours within the urogenital tract, challenging conventional classifications and diagnostic paradigms. We will examine the characteristics of these entities, exploring their clinical implications and prognostic significance. But we cannot forget the past…time-tested urogenital tumours continue to intrigue researchers and pathologists alike. Through a retrospective lens, we will revisit classic tumours, in light of contemporary evidence and new prognostic information. This presentation will also seek to introduce and elucidate grading systems for urogenital tumours, offering insights into their rational and potential implications for clinical practice. Join us as we navigate the complexities of tumour classification, charting a course towards improved diagnostic accuracy and enhanced oncologic patient outcomes.

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SPEAKER ABSTRACTS

Veterinary Pathology | Friday 30th August 2024, 11.00-12.00

HIGHLY INFECTIOUS DISEASES IN RUMINANTS - JUST THE USUAL SUSPECTS?

Angele Breithaupt

Ruminant livestock, including cattle, sheep, and goats play an important role in food security and nutrition, as well as in the livelihoods of farmers and others along the food chain. When it comes to relevant highly infectious or highly „contagious“ diseases in ruminants, some of you will probably first think of the „listed diseases“ published in the Terrestrial Animal Health Code of the World Organisation for Animal Health, WOAH [1] or national notifiable disease. Some may also consider emerging diseases based on recent publications or notification. The list of relevant diseases is far too long to be summarised in one overview lecture. My selection for cattle, sheep and goats for this overview is based on the following questions:

- In which of the WOAH-listed diseases did/does the diagnostic pathologist in Europe play a relevant role with regard to (re)discovery or routine diagnostics?
- Which emerging / re-emerging diseases should we as pathologists have “on our radar”?

The WOAH has defined clear criteria for the conditions under which a disease is included in the WOAH list: Briefly, in addition to the international spread of the pathogen on the one hand and the proven freedom (or impending freedom) of at least one country on the other, reliable means of detection and diagnosis must be available. A definition should clearly identify the cases. These criteria are supplemented by the requirements that, for example, natural transmission to and severe disease in humans is proven OR that there is a significant impact on the health of domestic animals. It is obvious that emerging diseases cannot be included in this list because essential information is missing.
Besides animal trade to and open borders within Europe, also climate change pose a challenge to pathologists in diagnostics. In addition to the “usual suspects”, we also have to keep an eye on diseases that occur in ruminants in our neighbouring countries, that might be transmitted by arthropods, or introduced by wild vertebrates. Some highly infectious diseases in ruminants are routinely checked, diagnosed and reported without the involvement of pathologists. Other diseases have not been present in Europe for a long time and the risk of introduction is assumed to be low. Thus, this presentation will try to cover relevant diseases that we can (ideally) clearly recognise or at least have a reasonable suspicion of. For these diseases, a brief overview of the current epidemiological situation and the typical pathomorphological changes will be summarized. If possible and appropriate, this is supplemented with the current state of scientific knowledge on pathogenesis and recommendations for final diagnosis.

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SPEAKER ABSTRACTS

Veterinary Pathology | Friday 30th August 2024, 12.00-13.00

EPIZOOTIC HEMORRHAGIC DISEASE: AN EMERGING VECTOR-BORNE VIRAL PATHOLOGY WITH A BIG IMPACT ON SPANISH CATTLE

Marcelo De las Heras
Universidad de Zaragoza, Zaragoza, Spain

Epizootic hemorrhagic disease (EHD) is a vector-borne viral pathology of wild and domestic ruminants, and a notifiable disease of the World Organization of Animal Health (WOAH) since 2008. It is caused by a virus of the Sedoreoviridae family and Orbivirus genus similar to those associated with Bluetongue or African horse sickness and transmitted by midges of the Culicoides genus. Seven serotypes have been described. We describe here the epidemiology and pathology of the disease in cattle with a particular focus on the last outbreaks in Spain. The first cases were notified in 2022 in the south of Spain and in the autumn of 2023 were distributed throughout the Iberian peninsula. Natural cases from adult and young cattle have been recorded and clinical pathology and anatomic pathology observations are summarized in this paper. All animals were positive for the EHD virus by PCR techniques. The clinical features in adult cattle were: edema on the head and eyelids; seromucous copious nasal discharge together with ptialism; epithelial loss, erosions, and petechiae on the oral mucosa and dental crown. A very striking characteristic was the appearance of the tongue hanging out of the mouth because the animal could not keep it inside. Suckler cows were more generally affected than dairy milk ones. Anorexia, coronary band, and hoves inflammation were also present. Heavy-weight animals remained on sternal recumbency unable to stand. In addition, some animals developed a severe clinical hemorrhagic picture with deads, of 24-48 of evolution after the first clinical signs were observed. In addition, veterinarians reported more fetal deaths, stillbirths, fetal malformations, weak calves, more problems during obstetric maneuvers, and very low-quality sperm on seminal assessments in affected herds. In calves for meat production (animals between 4-7 months old). Fists clinical signs observed were seromucous nasal discharge, dyspnea, fever maintained for a long time (21 days), coronary band-hoves inflammation, and stiff walk. On many occasions difficult to control with antiinflammatory drugs. Some animals showed a quick progression of the disease with high fever (40ºC) and signs of respiratory disease, inappetence, weakness, and death. After necropsy, similar lesions were observed either in cows or calves dying with the quick progression of the disease. Estimated mortality is around 1-2% of the animals. One of the striking features was the presence of hemorrhages in eyelids and another, when opening the skin, the presence of very fluid blood. Hemorrhages in the subcutaneous tissue and muscles, particularly in the head and neck, with some lymph nodes of this area completely hemorrhagic. The bloody pictures continued in the trachea, heart, and lungs. Small erosions or ulcers were found on the mucosa of the hard palate, esophagus, and abomasum. Hemorrhages were also frequent in intestinal serosal vasculature. Intense meningeal edema and hemorrhages were also added to gross findings. Few necropsied cows were pregnant and fetuses showed extensive hemorrhages under the skin and in thoracic and abdominal cavities and organs. The same changes were observed in stillbirth calves. The histopathological assessment showed a hemorrhagic pattern in most examined tissues and organs, but particularly intense in the lymph nodes, trachea, and lungs. Blood vessels (capillaries, small veins, and arteries) were mainly affected by endothelial cell tumefaction, detachment, or fading, together with thrombosis, disseminated intravascular coagulation, and perivascular hemorrhages. Early descriptions of the EHD in cattle in countries like the USA only reported transitory viremia with no clinical signs. However, in other geographic areas like Japan and several Mediterranean countries, pathology with severe clinical features sharing many similarities to the ones described in this paper was reported. There is no official impact assessment of the EHD to the Spanish bovine industry. Notwithstanding, economic losses have been estimated at 55 million euros in 2023 by farmer´s associations. Taking into account the data presented in this paper and from others, many concerns are emerging about the future evolution of the disease and its impact on the European bovine industry.

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SPEAKER ABSTRACTS

Veterinary Pathology | Friday 30th August 2024, 14.15-15.15

TOWARDS STANDARDIZATION OF HISTOLOGIC TUMOR PROGNOSTIC PARAMETERS - DOES IT REALLY MATTER?

Christof A. Bertram[1], Pompei Bolfa[2]
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Assessment of prognostic tumor parameters by diagnostic pathologists is critical for appropriate patient treatment decisions, which is reflected by the high research interest in this topic. However, there is a lack of consensus on the methodological standards for many tumor parameters in current research and a lack of guidelines for routine histological tumor evaluation. In this talk we give an overview of the current standardization efforts in veterinary pathology. The need for further standardization is exemplified by discussing critical points of developing, reporting and validating histologic tumor grading systems following recommendations by a recent guideline (VCGP.org).

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SPEAKER ABSTRACTS

Veterinary Pathology | Friday 30th August 2024, 16.15-17.15

HUMANS FORENSICS -BRAIN TRAUMA

Joao Pinheiro
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Brain trauma is a vast and complex issue either in Clinical or Forensic Medicine. Its assessment requires a high skill of expertise and experience, regarding the different mechanisms of wounding that may be implied: blunt, incised, perforating or a combination of these. The shape of the external injuries produced by each of these tools are different and need to be distinguished during autopsy. The same applies for internal injuries, from the scalp, through the bone, till we reach the intracranial structures. We will begin with a conceptual schema about the objectives of the forensic autopsy. Following, we'll briefly introduce the blunt typical injuries: abrasions, echimosis and lacerations, using several images. The incised (where length is dominant) and perforating injuries (depth is the main dimension) will also be presented with key elements which discriminate them from the previous. Finally, an approach to gunshot wounds will be given, and its features, regarding the type of weapon, the kind of ammunition, the range of discharge of the gun, and many other questions that arise with this type of weapon. Technical autopsy tips to observe these external wounds will be provided. Internally, the findings within the soft subepicranial tissues will be explained and its relation, both with the external and with the skull and endocranial injuries. Concerning the latter, special attention will be put on the meningeal haemorrhages-epidural, subdural and subaracnoid. Brain lesions, like contusion foci, cerebral lacerations, cerebral hematomas, brain stem haemorrhages, cerebral oedema and more, will be largely presented and documented, as well as histologic alterations, such as diffuse axonal injury. Elements to stablish the difficult differential diagnosis between spontaneous brain haemorrhage and a traumatic one will be given. All the matters will be profusely illustrated and integrated in the story of real cases, which will invite the audience to participate in the resolution of some criminal cases. We will finalize with a brief reference to how to write a medicolegal report, with special attention to its conclusions.

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SPEAKER ABSTRACTS

Toxicological Pathology | Saturday 31st August 2024, 09.00-09.45

COMPUTATIONAL PATHOLOGY APPLIED TO TOXICOLOGIC PATHOLOGY: LEARNING TO RIDE AI'S WAVE OF OPPORTUNITY

Julie Boisclair
Novartis, Basel, Switzerland

The field of artificial intelligence is progressing very rapidly. How can we, as toxicologic pathologists, keep up with this innovation wave? The presentation is about how to use computational pathology to enhance the toxicologic pathology field with artificial intelligence (AI) tools. The presentation shows the factors and conditions that help our AI transformation, emphasizes the need for a multidisciplinary collaboration and the role of the pathologist in AI model creation. Then it explores the different kinds of AI models and methods that can serve different goals in toxicologic pathology. The presentation ends by imagining the future of the toxicologic pathologist and the path to validation in the light of AI innovation and transformation.

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SPEAKER ABSTRACTS

Toxicological Pathology | Saturday 31st August 2024, 10.00-10.45

NEUROPATHOLOGY INSIGHTS VIA CRYOFLUORESCENT TOMOGRAPHY (CFT)

Elizabeth Galbreath
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One area where pathologists can augment the effectiveness of drug development teams is through identifying tissue and cellular deposition, and distribution of therapeutics in the context of histopathology. Frequently ADME/DMPK tissue distribution studies are conducted using either selective tissue dissection followed by homogenization, or quantitative whole-body autoradiography (QWBA) analyzed by phosphor imaging. Although selected tissues can be evaluated using matrix-assisted laser desorption/ionization imaging mass spectrometry in conjunction with a pathologist to identify cellular localization of a therapeutic, it is not a feasible approach for whole animal assessment. Whole animal assessment by QWBA lacks the resolution to provide information on the localization of the therapeutic in specific cell types. Cryo-Fluorescence Tomography (CFT) is a transformative 3D imaging technique that provides whole body anatomical and fluorescence images at micron level resolution. Images can be overlaid for enhanced visualization of on-and off-targeted drug biodistribution and protein expression with the ability to collect tissue sections for secondary analysis. This presentation will cover examples of whole body ASO and AAV biodistribution, including insights on CSF drainage pathways, detection of AAV distribution, and AAV-directed gene expression in unexpected tissues/cell types.

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SPEAKER ABSTRACTS

Toxicological Pathology | Saturday 31st August 2024, 11.30-12.15

TISSUE TECHNOLOGY IN BLOOD-BRAIN BARRIER ORGANOIDS AS COMBINED EFFICACY/TOXICITY HIGH THROUGHPUT SPATIAL READOUTS IN EARLY DRUG SCREENING

Luisa Bell
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Complex in vitro models (CIVM) offer an unprecedented opportunity to support enhanced preclinical to clinical translation by generation of high-quality preclinical mechanistic data in human systems. As an example, validated in vitro models of the blood-brain barrier (BBB) could facilitate effective testing of drug candidates targeting the brain early in the drug discovery process. However, adapting the standard histopathology workflows to CIVM is challenging due to their small size, differences in culturing devices, and cell composition/origin compared to animal tissue evaluation.

We have developed different histotechniques using BBB organoids to support model engineering, model characterization and compound distribution as well as efficacy/toxicity evaluation in early drug screening. First, our histo-embedding technique allowed immunohistochemistry (IHC) - and multiplex immunofluorescence-guided characterization with spatial evaluation of cell types. Second, histo-embedding enabled spatial evaluation of compounds via Matrix Assisted Laser Desorption/Ionization mass spectrometry (MALDI MS) and IHC for both small and large molecules, respectively. Third, to add on the standard in vitro cell death assessment (5x standard Caspase assay), our histotechniques and digital workflow using Hematoxylin and Eosin (HE) whole slide scans allowed histopathological evaluation of single cell toxicity. We developed a high resolution (40x), single cell morphologic artificial intelligence (AI) algorithm to detect necrotic/apoptotic cells on HE scans. As a proof of principle, we used Staurosporine to induce cell death in BBB organoids and validated the performance of our algorithm towards pathologists' evaluation. Compared to the 5x standard Caspase assay, our AI morphological readout adds single cell and spatial resolution, digitalization, automation, high throughput and reproducible components giving additional insights on the necrotic effect on specific cell types and mechanism of action.

Overall, our developed histotechniques allow additional readouts for assessing efficacy and toxicity in a reproducible, robust, sensitive and high-throughput manner in preclinical drug development. Our developed label-free end-to-end digital AI readout will accelerate decision-enabling experiments in preclinical drug development and in the future, can be transferred to other organoid models.

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SPEAKER ABSTRACTS

Toxicological Pathology | Saturday 31st August 2024, 12.15-13.00

UNVEILING CNS COMPLEXITY AND DRUG RESPONSES THROUGH SPATIAL TRANSCRIPTOMICS

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A detailed understanding of disease pathogenesis, target expression, as well as efficacy and safety parameters is essential for the successful development of central nervous system (CNS) targeting compounds. In this regard, spatial transcriptomics (ST) represents a revolutionary technology that allows for the visualization and quantification of transcriptomic profiles in the context of tissue morphology. ST encompasses a plethora of commercially available technologies that are of interest for pharmaceutical companies. ST platforms are categorized as sequencing or imaging based technologies[1]. Imaging based methods use panels for hundreds or several thousands of mRNA targets and provide subcellular resolution[2-3]. Sequencing based approaches cover the full transcriptome, however the resolution is limited by the designed DNA array used in the respective technology (range ~8µm-100µm)[4]. Most platforms entail the possibility to map the transcriptome signatures to the HE slide or to combine the ST readout with immunofluorescent protein staining. This provides pathologists the unique opportunity to correlate histomorphological changes or compound distribution patterns with transcriptomic signatures.
In this presentation, we will provide an overview of different ST platforms and delineate the role of the toxicologic or discovery pathologist in experimental setup, data analysis and interpretation. We further exemplify across therapeutic modalities and preclinical animal models, how spatial transcriptomics can support the development of CNS-targeting compounds[5-6].

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SPEAKER ABSTRACTS

Veterinary Pathology | Saturday 31st August 2024, 11.30-13.00

THE GLOBAL HEALTH PATHOLOGY NETWORK (GHPN) AND ESTABLISHING RELATIONSHIPS THROUGH FOUNDATIONAL PATHOLOGY TRAINING: AN INFORMATION SESSION

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The Global Health Pathology Network (GHPN) was started in 2015 at the request of the American College of Veterinary Pathologists as an organization to engage veterinary pathologists in Global Health.[1] Since 2016, the GHPN operates under the umbrella of the CL Davis-SW Thompson DVM Foundation (https://ghpn.cldavis.org/). The network focuses on three main areas and conducts workshops in locations with limited resources.

Focus areas of the GHPN
1. Provide shared informational and guidance forums to facilitate meaningful international engagement and support outreach by educational programs.
2. Facilitate self-directed connections in the network that may lend to regional/country-specific groups for joint capacity building.
3. Use the network expertise to respond to requests for ground work pathology training and basic work in less developed countries.

Workshop organization and philosophy
A researcher, professor, and/or veterinarian from a university in a host country contacts the GHPN through the website. Following this initial outreach (typically 6-8 months prior to the tentatively proposed workshop date), an introductory discussion occurs between the host faculty member and the GHPN via a remote online platform and/or email to determine feasibility of and scope for a training workshop. Pending feasibility, a GHPN workshop training team, consisting of 1-3 network members and the vital host faculty member, is identified and formal planning talks begin. Leading up to the workshop, there are scheduled online meetings with the host faculty member to obtain input on goals, objectives, and, most importantly, diseases of interest for workshop attendees. Notably, general workshop topics include basic mechanisms of disease, postmortem examination (necropsy) techniques, sample collection particularly under field conditions, and lesion description and interpretation. The workshops also emphasize recognition, reporting, and control of country-specific diseases many of which have implications in economy, trade, and animal, human, and environmental health. The workshops are designed to be dynamic and interactive, using case-based scenarios incorporating different aspects of foundational pathology training. In addition, prior to conducting the workshop facilitators are expected to learn and have basic working knowledge of the local veterinary and livestock sector, economy, political system, history, culture, and language of the host country. Workshops have a duration of 3 to 4 days and adult learning principles with interactive modalities are used. Group discussion of case scenarios, problem-solving exercises, and pathology-oriented games are part of the interactive training. Active participation and two-way communication between facilitators and attendees are essential, and occur through participant engagement and peer-to-peer discussion, flip chart utilization, and other engaging platforms. Although projected material (i.e., PowerPoint) is used at

SPEAKER ABSTRACTS

times, very limited time is dedicated to formal didactic lectures, as the intent is to encourage collaborative thoughts, mutual problem solving, and “aha” moments. Necropsy wet labs with species of interest focused on sample collection and gross report writing are conducted during 1 or 2 half-days. As a critical member of the training team, the host faculty member always participates as a facilitator of the workshops.

Past workshops and outcomes

Partnering with local universities, the GHPN has conducted workshops in Bangladesh, China, Ethiopia, Guatemala, Indonesia, Kenya, Morocco, Namibia, Nepal, Pakistan, Rwanda, Serbia, and Uganda. There is a very active collaborative node at the University of Veterinary and Animal Sciences (UVAS) in Lahore, Pakistan, with yearly workshops and monthly online sessions being conducted. Workshop participants often vary in educational and professional background and/or stage in training providing a unique aspect to each workshop that often has to be customized. In general, participant profiles include the presence of two or more: academic veterinarians (including veterinary pathologists and/or other disciplines), national animal health services veterinarians, field veterinarians, laboratory diagnosticians and technicians, graduate and postgraduate students, and paraveterinary workers.
At the end of the workshop, participants are asked to evaluate the organization, facilities, and instruction on a 1-5 scale. Input on “Likes” and “Dislikes” as well as items that should be included (or excluded) from future workshops are part of the post-workshop evaluation process. This important feedback is used to make improvements in future workshops and decide on new topics.

How to get engaged

The ultimate goal of the GHPN is to connect people and locations around the world on the basis of foundational pathology. As such, veterinary pathologists from all areas of expertise, pathology residents, and students are welcome to become part of the network. For that, individuals or institutions can submit their data, region/s of interest and other queries through the website (https://ghpn.cldavis.org/get-engaged/)

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SPEAKER ABSTRACTS

Veterinary Pathology | Saturday 31st August 2024, 11.30-12.15

PARR: WHAT DOES IT MEAN?

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Distinguishing lymphoma from reactive lymphocyte proliferation based solely on histopathology can pose challenges in certain cases. While immunohistologic examination can clarify some of these cases, others remain ambiguous. Clonality analysis, also known as PARR (PCR for Antigen Receptor Rearrangement), takes advantage of the clonality of lymphomas. This means that neoplastic lymphocyte populations originate from a single transformed cell, whereas reactive lymphocyte populations are recruited from different cells.

During maturation, lymphocytes undergo gene rearrangements for antigen receptors (B-cell receptor, T-cell receptor), which involves combining various segments of antigen receptor genes from encoded variants in the genome (V(D)J recombination). Random nucleotide insertions and deletions occur at the junction sites during this process, leading to differences in both base composition and length of the antigen receptor genes. Neoplastic lymphocyte populations exhibit identical antigen receptor gene patterns, thus representing a (mono)clonal population. Conversely, reactive lymphocyte populations display variability in corresponding regions, indicating a polyclonal population. PCR amplification of regions with length variability allows for distinguishing neoplastic and reactive lymphocytes. Capillary electrophoresis reveals uniform PCR products with distinct peaks for neoplastic lymphocytes, contrasting with a nearly normal distribution of peaks for reactive lymphocytes, akin to a Gaussian curve. However, various factors can adversely affect the test results of this ideal scenario complicating the interpretation. For example, the type and preparation of the original material can significantly impact the feasibility and interpretation of the test. In addition, the selection of a primer set from various primer sets published in veterinary medicine influences the sensitivity and specificity of the test. The phenomenon of pseudoclonality, characterized by the random amplification of individual targets in the presence of few lymphocytes in the sample or poor DNA quality, must be considered during PCR setup. In addition, changes in primer binding sites, such as those resulting from somatic hypermutation play an important role, especially in B cells. Furthermore, the occurrence of biclonality, where antigen receptor genes may undergo recombination on both alleles, must be considered during result evaluation.

In conclusion, interpreting electrophoresis results is not always straightforward and requires a solid background knowledge. Hence, the overall assessment should always involve a combination of all available data, including histopathology, immunohistology, clinical findings, and clonality analysis.

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SPEAKER ABSTRACTS

Veterinary Pathology | Saturday 31st August 2024, 12.15-13.00

FROM PIXELS TO PATTERNS: THE TRANSFORMATIVE POWER OF AI IN NEUROPATHOLOGY RESEARCH

Lev Stimmer
Paris Brain Institute, Paris, France

Artificial intelligence stands at the forefront of neuropathological research, offering innovative methods for the quantification of lesions across a spectrum of diseases, thereby enabling a nuanced understanding of pathological mechanisms. This talk delves into the transformative power of AI in neuropathology, elucidating how it assists the pathologist in identifying patterns and quantifying lesions that may be overlooked by traditional analysis.
Through the lens of specific examples, such as the quantification of neuropathological changes like spongiosis in a mice prion model, or the segmentation and morphometric analysis of microglia, we highlight the critical role of AI in these intricate tasks. AI-driven algorithms can assist the pathologist by annotation of complex structures within histological sections that are densely populated or exhibit ambiguous boundaries. For instance, the segmentation of phosphorylated tau positive neuritic plaques and neurofibrillary tangles, which are emblematic of Alzheimer's Disease (AD) pathology, demonstrates that AI can significantly improve the annotation quality of complex neuropathological leasions, thus supporting the development of highly accurate models for the quantification of these histological biomarkers in AD brains.
While AI significantly enhances our analytical capabilities, the talk also addresses the inherent limitations of these quantitative approaches and emphasizes the necessity for pathologists to maintain a critical perspective when integrating AI into their research. This critical examination ensures that AI serves as a robust tool in the pathologist's arsenal, augmenting rather than replacing the nuanced insights provided by human expertise.

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CASE PRESENTATIONS

Toxicological Pathology | Thursday 29th August 2024, 17.45-18.00

YOU'VE THOUGHT OF IT, BUT HAVE YOU EVER SEEN IT? - INTERESTING NEUROPATHOLOGICAL FINDINGS IN THE BRAINS OF WISTAR RATS

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In an Extended One-Generation Reproduction Toxicity Study performed according to OECD guideline 443, the test substance was administered via the drinking water to male and female Crl:WI(Han) rats. A whole organ spectrum was investigated by light microscopy, including the Developmental Neurotoxicity (DNT) Cohorts of offspring animals. In F0 parental animals, the weights of the thyroid glands were increased in both sexes of the high dose group. Animals of the F1 generation showed a reduced terminal body weight in both sexes besides a weight increase and enlargement in thyroid glands in mid and high dose test group. Presented are direct and indirect findings in different organs in parental and offspring animals followed by a discussion of possible mode of actions.

In the thyroid glands, hypertrophy/hyperplasia of follicular cells was present in both sexes of the F0 and F1 generation. In the F0 generation, both sexes were affected in the low, mid, and high dose groups, showing a dose-dependent increase in severity. In the high dose group, 25/25 male and 21/25 female animals were affected, with the hypertrophy/hyperplasia graded mainly moderate to massive in male animals and slight to marked in female animals. In the F1 generation, the mid and high dose groups showed hypertrophy/hyperplasia of follicular cells in both sexes, with a dose-dependent increase in severity being present. In both generations, mineralization of colloid showed a dose-dependent increase in the affected dose groups in both sexes. Changes in thyroid hormone levels (decreased T4, increased TSH) were present in both sexes of the mid and high dose groups of both generations. The findings in the thyroid glands were considered a direct effect of the test substance.

In the hippocampus, heterotopic neurons were present in 7/20 male and 4/20 animals of the high dose group of the F1 generation. Hippocampal pyramidal neurons were scattered around their anatomically correct location in the stratum pyramidale, primarily affecting the CA2 and CA3 region, and to a lesser extent the CA1 region. These changes were also present and more distinctly visible in the Developmental Neurotoxicity Cohorts for weanlings and adults of the F1 generation for which animals were sacrificed on PND 22 and 77, respectively. Similar findings were described in publications noting observations after thyroid hormone disturbance (Shibutani et al 2009, Mano et al 1987). Hence, the proliferative changes of the thyroid glands with changes in hormone levels in F0 parental animals and/or a direct effect of the test substance on the F1 generation thyroid glands could be underlying mechanisms, potentially leading to a disturbance of neuronal migration.

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CASE PRESENTATIONS

Toxicological Pathology | Saturday 31st August 2024, 10.45-11.00

COMPARATIVE AAV-AMIR-SOD1-RELATED FINDINGS IN NERVOUS SYSTEM OF CYNOMOLGUS MONKEYS, NEW ZEALAND WHITE RABBITS, AND C57BL/6 MICE

I. Pardo[1], B. Grubor[1], C. Tremblay[2], K. Henry[1], Z. Hawley[1], P. Clarner[1], E. Tien[1], J. Lo[1]
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Histopathology findings in the dorsal root ganglia (DRG), trigeminal ganglia (TG), spinal cord and nerves associated with administration of Adeno-associated virus vectors (AAV) have been reported in Cynomolgus monkeys (NHP), but less commonly in other species, such as New Zealand white rabbits and C57BL/6 mice. We compared the histopathology findings in these 3 preclinical species at 4 or 6 weeks after a single lumbar intrathecal (LIT) or intracisterna magna (ICM) injection of AAV encoding an artificial miRNA targeting superoxide dismutase 1 (AAV- amiR-SOD1). The amiR sequence targeted SOD1 in NHPs and mice but not in rabbits. In one 6-week study, 2.5- to 3.5-year-old, male and female NHPs were dosed in the LIT space L4/L5 with 1.5x1014 or 3.0x1014 GC/dose of AAV-amiR-SOD1. In a 6-week study, 6-7-week-old male and female mice were dosed in the LIT space 6 with 8.0x1011 or 1.6x1012 GC/dose of AAV-amiR-SOD1. In a 4-week study, male rabbits were dosed with 8.31E12 to 1.14E13 GC/dose of AAV- amiR-SOD1 in the ICM. AAV-related histopathology findings in NHP, rabbit and mice were all characterized by degeneration/necrosis of neurons associated with mononuclear cell infiltrate (MCI) in the DRG and TG, and nerve fiber degeneration in the dorsal funiculi (of the spinal cord, dorsal nerve roots, sciatic nerve, saphenous nerve (mice only), trigeminal nerves (attached to TG). DRG findings were of greater incidence and severity in the lumbar regions than cervical and thoracic regions. The severity of the nerve fiber degeneration in nerves and spinal cord differed bilaterally. We observed 3 major histopathologic variations among these species. First, MCI reaction associated with neuronal changes in the DRG was not as prominent in mice as it was in NHP and rabbit, but an increased dose in mice resulted in slightly more severe lesions. Second, the presence of vacuoles in neurons undergoing degeneration/necrosis in the DRG and TG, as well as nerve fiber degeneration in the lateral funiculi, were typical findings in rabbits. Third, rabbits tend to develop more severe lesions in the TG compared to NHPs, while mice have not to date been reported to develop TG lesions. ICM injection might be associated with severity of TG in rabbits. In conclusion, NHPs, rabbits and mice develop similar AAV-related lesions in target tissues with some variations in severity, neuroanatomic predilection, and pathology features that could provide insights of different mechanisms of toxicity and the translatability of these findings to humans.

CASE PRESENTATIONS

Toxicological Pathology | Saturday 31st August 2024, 13.00-13.15

BILATERAL BASAL NUCLEI VACUOLAR LESIONS - A NOVEL AND EMERGING POTENTIAL BACKGROUND FINDING IN BEAGLE DOGS

S. Arms[1], S. Cahalan[2], F. Goulet[3], K. Hempel[1], G. Pellegrini[2], S. Rau[1], J. Schlichtiger[1], T. Nolte[1]
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Degenerative lesions specific to the basal nuclei have not been previously reported as a background finding in Beagle dogs. Bilateral vacuolar lesions in the caudate nucleus, and less frequently the putamen, have emerged in individual Beagle dogs (total of 29 cases) utilized in nonclinical studies, regardless of the respective dose group and in various institutions worldwide. Demographics of affected dogs, histopathology, terminology, etiological hypotheses, and interpretation of the lesions in the context of nonclinical safety studies will be discussed.

CASE PRESENTATIONS

Toxicological Pathology | Saturday 31st August 2024, 13.15-13.30

ULTRASTRUCTURAL CHANGES IN SENSORY NERVES: FINDINGS IN DRG AND PERIPHERAL NERVES

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Dog aged 1-2 years
Rats aged 10-16 weeks
Toxicity studies with undisclosed compound with inlife clinical signs of peripheral nerve alteration. Presentation of ultrastructural microscopy for interactive learning.

Introduction: Evaluation of peripheral nerves using resin embedding and/or ultrastructural techniques provides additional tools and levels of resolution that can help characterize the findings observed at light microscopy.

Methods: Using established laboratory methods, appropriately collected and fixed samples of peripheral nerves from rodents, dogs or nonhuman primates or dorsal root (spinal sensory) ganglia from rodents were trimmed, processed, embedded in resin and either stained and examined with toluidine blue or, further trimmed, ultrathin sections and evaluated via transmission electron microscopy (FEI) to characterize a variety of light microscopic findings observed by routine hematoxylin and eosin stained, paraffin embedded sections.

Results: Examples of normal, common artifacts and test article-related findings will be presented, compared and described.

Conclusion: The impact of resin-embedded peripheral nerve microscopic evaluation, supplemented when appropriate with ultrastructural transmission electron microscopy can provide additional information to inform risk assessment in drug development.

POSTER FLASH

Veterinary Pathology | Thursday 29th August 2024, 09.45-09.51

EVALUATION OF ALTERNATIVE IMMUNIZATION STRATEGIES AGAINST PARATUBERCULOSIS IN GOATS

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Background

Vaccination stands as the most cost-effective approach for controlling ruminant paratuberculosis, caused by Mycobacterium avium subspecies paratuberculosis (Map) and characterized by a chronic granulomatous enteritis. However, due to the limitations of current subcutaneous vaccines, this study aims to evaluate the efficacy of alternative routes of immunization in goats.

Materials & Methods

Goat kids (n = 6 per group) were immunized with whole-cell, heat-inactivated, 316F Map strain vaccines through different routes: oral, Quil-A® adjuvanted (OV), intradermal (IDV) and subcutaneous (oil-based adjuvanted, Gudair®) (SCV). Non-vaccinated animals (NV) (n = 10) were used as control. After one month, three and seven animals from each vaccinated and non-vaccinated group, respectively were challenged with a low passage type C field isolate. Peripheral immune response and Map fecal shedding were analyzed throughout the study. Finally, tissue bacterial load and histological lesions were assessed, 11 months after vaccination.

Results

OV and IDV failed to induce significant humoral responses and only evoked mild and delayed cellular immune responses compared to SCV. Despite this, all challenged groups developed granulomatous lesions, some of them severe. Although OV showed a reduction in granuloma counts, neither it nor the IDV decreased bacterial load, in contrast, SCV demonstrated superior efficacy, reducing both lesions and Map fecal shedding.

POSTER FLASH

Veterinary Pathology | Thursday 29th August 2024, 09.51-09.57

EXTRACELLULAR VESICLE ISOLATION AND CHARACTERIZATION FROM BOTTLENOSE DOLPHIN'S (TURSIOPS TRUNCATUS) BLOW

V. Moccia[1], C. Centelleghe[1], A. Zendrini[2,3], G. Pesce[4], E. Alvarez[4], E. Esposti[4], A. Radeghieri[2,3], P. Bergese[2,3], S. Mazzariol[1], V. Zappulli[1]

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Background
The bottlenose dolphin (Tursiops truncatus) is a species of interest for Mediterranean Sea health monitoring. Despite in vivo sampling is arduous, collecting and analyzing cetacean's blow is an innovative and non-invasive monitoring alternative. Extracellular vesicles (EVs) are membranous nanostructures released by cells and have been widely studied in human and veterinary medicine as diagnostic biomarkers. Aiming to further apply blow derived-EVs as tools to monitor dolphin health, here we preliminary describe EV-isolation and characterization from bottlenose dolphin blow.

Materials & Methodss
Blow samples were collected using a six-well petri dish from four bottlenose dolphins kept under human care. Samples were dissolved in PBS (4.5 mL) and ultracentrifuged (120,000 x g for 90 minutes) to collect the EV-enriched pellet. EVs were then analyzed for particle size distribution and concentration with Nanoparticle Tracking Analysis (NTA), for protein expression with Western Blotting (WB) and visualized and measured with Atomic Force Microscopy (AFM).

Results
NTA detected particles in the size range of EVs (40-600 nm) with a different concentration in each sample (from $7.7*10^{10}$ to $2.5*10^{12}$ particles/ml), data confirmed by AFM showed the presence of EV-like structures with a diameter compatible with the NTA determinations. At WB, all EV-pellets were positive for EV-markers (CD9, integrin-beta) and negative for OmpA, a Gram-negative bacterial membrane protein, and for the negative control Calnexin.

Conclusion
We report for the first time EV-isolation and characterization from bottlenose dolphin's blow. Analysis of blow derived-EVs might be exploited for health status monitoring of both captive and free-ranging cetaceans.

POSTER FLASH

Veterinary Pathology | Thursday 29th August 2024, 09.57-10.03

METHODOLOGY: MASS SPECTROMETRY-BASED IDENTIFICATION OF ANIMAL AMYLOIDOSIS

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Background
Amyloid fibrils are often difficult to identify by immunohistochemistry because they have abnormal structures compared with normal proteins and are often composed of truncated proteins. Minor animal species can also cause problems with antibody cross-reactivity. In addition, even when immunohistochemistry works well, it is sometimes difficult to determine whether the immunoreaction is derived from amyloid or other coexisting proteins. This study aimed to use mass spectrometry to identify animal amyloidosis.

Materials & Methods
Formalin-fixed paraffin-embedded tissues with severe amyloid deposition, including mammary tumors of five dogs, kidney of one cottontop tamarin, kidneys of three lions, kidneys of 15 Japanese squirrels, and thyroid C-cell carcinoma of one cat, were used for analysis. Amyloid deposits were collected from Congo red-stained tissue sections manually or by laser microdissection, solubilised in a surfactant, digested by trypsin or chymotrypsin, and subjected to liquid chromatography-tandem mass spectrometry. The data were analysed using the Mascot Server to identify the constituent proteins in the amyloid deposits.

Results
Using this method, we have identified α-S1-casein (dog), apolipoprotein A-IV (cottontop tamarin), apolipoprotein C-III (lion), fibrinogen α-chain (Japanese squirrel), and calcitonin receptor-stimulating peptide 1 (cat) as novel animal amyloid precursor proteins. Analysis of the frequency of peptides detected by mass spectrometry allowed the prediction of amyloidogenic regions in amyloid precursor proteins.

Conclusion
Mass spectrometry was effective in identifying amyloid precursor proteins in animals that were difficult to identify by immunohistochemistry, and also enabled the evaluation of changes within the amyloid precursor proteins.

POSTER FLASH

Veterinary Pathology | Thursday 29th August 2024, 10.03-10.09

MULTISYSTEMIC MYXOZOAN INFECTION IN TOADFISH, TETRACTENOS HAMILTONI CAUSED BY MONOMYXUM INCOMPTAVERMI

A. Nakashima[1], **N. Mishra**[1]
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Background
Myxozoan infections in fish typically affect organs like gills and spinal cords, particularly in Salmonids. This study presents a unique case of multisystemic myxozoan infection in a toadfish (Tetractenos hamiltoni) from Connecticut, USA.

Materials & Methods
A 10-year-old toadfish (Tetractenos hamiltoni) was submitted to the Connecticut Veterinary Medical Diagnostic Laboratory (CVMDL) for postmortem examination. The fish exhibited anorexia, cloudy eyes, oral cavity discoloration, and skin necrosis. Tissue sections from various organs were stained using standard techniques like Hematoxylin and Eosin (H&E), as well as special stains such as Twort's Gram stain, Grocott's methenamine silver stain, Wright-Giemsa stain, Ziehl-Neelsen acid-fast stain, and Periodic Acid Schiff (PAS) stain. Skin samples were sent to the University of Florida Zoological Medicine Diagnostic Laboratory for Myxozoa PCR.

Results
Postmortem examination revealed a 3.0 cm x 3.0 cm white discolored area with erosion and ulceration on the head, cloudy eyes, and white nodules on the left gill. The liver was diffusely pale. Histopathology showed myxozoans mixed with necrotic debris in the skin, liver, and eyes. Organisms stained partially positive with Giemsa stain but not with Gram stain, Grocott's silver stain, Ziehl-Neelsen stain, or PAS stain. PCR identified Myxosporea, with 92.2% homology to Monomyxum "MF-2015" and 90.4% to Monomyxum incomptavermi.

Conclusion
This study reports the first case of multisystemic myxozoan infection in toadfish caused by Monomyxum incomptavermi, diagnosed by CVMDL. This finding emphasizes the importance of monitoring and managing infectious diseases in aquatic populations due to their zoonotic potential.

POSTER FLASH

Veterinary Pathology | Thursday 29th August 2024, 10.09-10.15

COMPARATIVE ANATOMY AND HISTOLOGY OF GUSTATORY PAPILLAE IN LABORATORY ANIMAL SPECIES

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Background
Taste buds are the central unit of the gustatory sensory system. In the tongue, they are grouped in gustatory papillae. More than 200 medications can induce taste disorders in human patients. However, the impact of drug candidates on the gustatory system are usually poorly evaluated in nonclinical studies. The aim of this work was to illustrate and compare the anatomy and histology of gustatory sense organs in laboratory animal species.

Materials & Methods
Animal species included Cynomolgus macaque, Beagle dogs, Göttingen minipigs, New Zealand white rabbits, Wistar Han rats, and CD1 mice. The tongue of four young mature animals per species (two males and two females) was sampled. Macroscopic evaluation of the tongue was performed with a digital microscope (Keyence VHX-2000E). Routine stains (Hematoxylin-Eosin, Masson's Trichrome), immunohistochemistry (NF200 and S100) and TEM were performed.

Results
There were notable inter-species differences in the number, size, and (to a lesser extent) distribution of gustatory papillae throughout the tongue (e.g., Vallate papillae were in the posterior tongue in all animals, but their number and size was lower in rodents than non-rodent species). Taste buds were located in the lateral aspect of vallate and foliate papillae and in the apical aspect of fungiform papillae.

Conclusion
Gustatory sensory system differs among laboratory animal species. The number and location of tastes buds notably differed among each type of papillae and slightly among animal species. This comparative illustration of the morphological features of gustatory papillae can assist in future studies of taste disorders.

POSTER FLASH

Veterinary Pathology | Friday 30th August 2024, 09.45-09.51

FROM CONCEPT TO REALITY: THE JOURNEY OF ESTABLISHING THE AUSTRALIAN COMPANION ANIMAL REGISTRY OF CANCERS (ACARCINOM)

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Background
Establishing veterinary cancer registries represents a critical step for providing comprehensive epidemiological cancer information that otherwise would remain fragmented and sparse or limited in scope. This abstract provides insight into the development and implementation of an Australia-wide cancer registry aimed at the systematic collection, curation and storage of nationally representative feline and canine cancer cases.

Materials & Methods
ACARCinom harvests feline and canine cancer data from veterinary pathology laboratories of 4 veterinary schools (UQ, University of Sydney, Murdoch University, University of Adelaide) and 2 major diagnostic services (IDEXX, Gribbles). Using an approach common in object-oriented programming, an automated converter for each data source provider was developed and tested.

Results
Since January 2024 more than 7000 coded records have been processed. Outcomes of the data parsing algorithm for extracting and transforming raw cancer diagnosis data include: 1) finding the relevant text in the report to begin analysis; 2) parsing the report text through data dictionaries (diagnosis, site of the tumour, certainty levels, metastasis information); 3) result presentation after marking up the report text with HTML tags; 4) result storage, processing and quality control.

Conclusion
ACARCinom's capacity to process thousands of reports per minute minimises human intervention in diagnostic classification. Decades of historical and prospective cancer data can now be processed and made available for diagnostic and research purposes. The platform is continuously evolving to improve data quality, expand geographical coverage, and incorporate more data providers.

POSTER FLASH

Veterinary Pathology | Friday 30th August 2024, 09.51-09.57

PORCINE NEPHROBLASTOMA AS A SPONTANEOUS ANIMAL MODEL FOR WILMS'TUMOR: PRELIMINARY RESULTS

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Background

Wilms' tumor (WT) is the most frequent renal tumor in children. Treatments consist of surgical removal of the tumor before or after chemotherapy but some patients develop recurrence or chemotherapy adverse effects. Thus, the availability of a possible animal model could be helpful for a better knowledge of WT and for the development of new therapeutic strategies. Therefore, the purpose of this first study, in the framework of a national research project (PRIN 20227FX7KM) including investigators in human and veterinary medicine, is to histologically characterize porcine nephroblastoma to validate it as a spontaneous animal model for WT.

Materials & Methods

Hematoxylin-eosin-stained slides of 31 cases of porcine nephroblastoma were retrieved from the archive and evaluated, by human and veterinary pathologists according to the"UMBRELLA SIOP-RTSG 2016 protocol" for the presence and the percentage of the 3 typical Wilms tumor components (epithelial, blastemal, and stromal) and for the degree of anaplasia.

Results

Specifically, cases were classified as subtypes: epithelial, blastemal, stromal and mixed according to the percentage of presence of each component. As a result of this classification, most of our tumors were classified as mixed (26/31), three were classified as blastematic and two as epithelial. Anaplasia was present, only focally in one case.

Conclusion

Most of the nephroblastoma examined were classified as mixed, reflecting what has been reported in human medicine The results obtained, which need to be corroborated by further immunohistochemical studies, suggest porcine nephroblastoma as a good animal model for human Wilms' tumor.

POSTER FLASH

Veterinary Pathology | Friday 30th August 2024, 09.57-10.03

STAT3 PATHWAY IN CANINE ORAL MELANOMA: A POTENTIAL THERAPEUTIC TARGET?

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Background
Canine oral melanoma is an aggressive tumor with limited therapeutic options and short survival times. Signal transducer and activator of transcription (STAT)3 is a regulator of transcription that can be constitutively activated in different types of cancer, but can also be activated by Janus kinases (JAK). Our aim is to assess the expression of pSTAT3 and JAK1 in a retrospective cohort of canine oral melanomas.

Materials & Methods
Forty-three cases of canine oral melanomas were retrospectively selected and immunohistochemistry was performed to assess the expression of pSTAT3 and JAK1. The expression of STAT3, JAK1, JAK2, and TYK2 was also assessed by RT-qPCR on three OM cell cultures. On 2D cell culture, the expression of pSTAT3, JAK1, and JAK2 was assessed by IF.

Results
Expression of pSTAT3 was observed in the nucleus of neoplastic cells, with variable percentages (1-80%) in all tested melanomas and cell cultures. Instead, JAK1 was observed in the cytoplasm of melanoma cells (50-95%) in all tested cases. Gene expression analysis confirmed highly expressed STAT3 and a higher expression of JAK2 among the tested Janus kinases.

Conclusion
Results from the present study show activation of the STAT3 pathway in canine oral melanoma, identifying it as a potential prognostic marker and an interesting therapeutic target.

POSTER FLASH

Veterinary Pathology | Friday 30th August 2024, 10.03-10.15

TILS, TLSS AND HLMS EVALUATION IN CANINE MAMMARY CARCINOMAS

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Background
The tumour microenvironment (TME) is a dynamic entity that involves different cell populations. Tumour-infiltrating lymphocytes (TILs) are composed of B, T and natural killer cells. TILs are considered an early stage of maturation of tertiary lymphoid structures (TLSs). TLSs are composed of lymphocytes, macrophages, mast cells and dendritic cells. Macrophages are commonly found in TME, but only recently hemosiderin-laden macrophages (HLMs) have been highlighted for their possible role in cancer progression.
This study aims to evaluate the presence and distribution of TILs, TLSs and HLMs in canine mammary carcinomas.

Materials & Methods
Canine mammary carcinoma samples (n=150) were evaluated using HE. Immunohistochemistry with CD21 antibody was carried out to mark dendritic cells. TILs, TLSs and HLMs presence were evaluated in stromal and peritumoral compartments. Stromal TILs were assessed using the International TILs Working Group guidelines. Klintrup score was adapted to evaluate peritumoral TILs.

Results
CD21-positive cells were in inflammatory cell groups densely packed and often organized in a follicular-like pattern recognized as TLSs. Most of the cases included in the study had a low percentage of stromal TILs. Peritumoral TILs were frequently present either as scattered cells or with a band-like disposition. The presence of TLSs and TILs appeared to be directly correlated. HLMs distribution was independent of histotype and tumour grading.

Conclusion
In the era of immunotherapy, TME should be given more attention. International TILs Working Group guidelines and Klintrup score can be easily adapted to canine mammary tumours, and CD21 can be useful to identify TLSs.

POSTER FLASH

Veterinary Pathology | Friday 30th August 2024, 10.15-10.21

PORCINE ALVEOLAR MACROPHAGES AND NASAL EPITHELIUM CAN INTERNALIZE PORCINE EPIDEMIC DIARRHEA VIRUS (PEDV) BUT DO NOT SUPPORT REPLICATION IN VITRO

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Background
The present study explored a potential alternative route of porcine epidemic diarrhea virus (PEDV) infection by examining the cellular kinetics in porcine alveolar macrophages (PAMs), 3D4/21 cells (3D4) and infecting nasal turbinate epithelium.

Materials & Methods
PAMs and 3D4 were exposed to PEDV (MOI 1). Viral load in cells and supernatants (SN) was analyzed at various hours post-inoculation (hpi) using RT-qPCR. Infectious virus in the cell culture was quantified by $TCID_{50}$/ml and immunofluorescence (IF). PEDV internalization was examined by IF at 24 and 48 hpi, with morphological alterations assessed using transmission electron microscopy (TEM). Proinflammatory/antiviral cytokines in SN were analyzed using ELISA and Luminex. Nasal turbinate organoids (NTOs) cultivated in 2D monolayer and air-liquid interface were exposed to PEDV (MOI 1). At 24 hpi, RT-qPCR and IF were conducted in cells and SN.

Results
PEDV RNA levels peaked at 12 hpi in both PAMs and 3D4, before gradually declining. Viral titer was low and showed no significant difference between cell types. Few PAMs and 3D4 tested positive for PEDV IF, with no increase between 24 and 48 hpi. TEM did not reveal virions or cell membrane rearrangements. No expression of proinflammatory and antiviral cytokines was detected in any cell type. NTOs showed similar PEDV RNA levels in cells and SN at 24 hpi, along with the positivity for PEDV IF.

Conclusion
PAMs, 3D4 and respiratory epithelium can capture and internalize PEDV, but they do not support viral replication. Moreover, they did not induce an antiviral or anti-inflammatory response.

POSTER FLASH

Veterinary Pathology | Friday 30th August 2024, 10.21-10.27

IMPACT OF HANDLING TECHNIQUES ON UMBILICAL ULCERATIONS AND OMPHALITIS IN PIGLETS

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Background
In conventional pig production, piglets can be caught and lifted in a single hindleg when handled by humans. It has been speculated that this practice may impede the healing of the umbilicus. The aim was to evaluate whether the handling technique has an impact on the healing of the umbilicus in piglets.

Materials & Methods
In a conventional sow herd, 103 litters were divided into two groups at birth. In group 1 (n = 52 litters, $\bar{x}$ = 19 piglets/litter), piglets were caught and lifted in one hind leg. In group 2 (n = 51 litters, $\bar{x}$ = 18 piglets/litter), piglets were caught and lifted under the abdomen. In both groups, piglets were handled at least 8 times within the first 14 days of life. Subsequently, 50 female piglets aged 14 days were randomly chosen from each group, euthanized and necropsied. Differences between the groups were determined using Fisher's exact test ($p \leq 0.05$).

Results
An umbilical ulceration was present in 42% and 52% of piglets in group 1 and 2, respectively. Omphalitis was present in 16% and 30% of piglets in group 1 and 2, respectively. None of the findings differed significantly between the groups. In the 23 piglets with omphalitis (group 1 and 2) lesions were in the umbilical arteries (n=15), vein (n=1), urachus (n=2) or inconclusive (n=5).

Conclusion
The handling technique did not impact the presence of umbilical ulcerations and omphalitis in piglets.

POSTER FLASH

Veterinary Pathology | Friday 30th August 2024, 10.27-10.33

CANINE ORAL MELANOMA MACROPHAGE-POOR HETEROSPHEROIDS: AN UPSIDE DOWN MODEL TO INVESTIGATE TUMOR MICROENVIRONMENT

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Background
Tumor immune microenvironment (TIME) comprises an intricate net of connections and interactions. Among the cellular populations in TIME, macrophages are one of the most represented, accounting in some cases for almost half of the cells present within the neoplastic lesion. Aim of this study is to set up and characterize a model to study canine oral melanoma-associated TIME, reducing, if not excluding, macrophages from the experimental in vitro model.

Materials & Methods
Spheroids were established from primary 2D-cell cultures from a canine oral melanoma. After isolation from whole blood of 4 dogs, peripheral blood mononuclear cells (PBMC) were incubated on a 12-well plate for 8 hours, to allow macrophage adhesion. Non-adherent cells were quantified and put in co-culture with canine oral melanoma spheroids. Part of the cells were set aside to assess phenotype by immunocytochemistry (CD18,CD3, CD20, CD4, CD8α, CD8β, IBA1 and CD204). After 3 days of co-culture, part of heterospheroids was included in paraffin using Cytomatrix® as a support, whereas another part was snap-frozen for characterization by immunohistochemistry and immunofluorescence.

Results
ICC revealed <1% of macrophages/monocyte after adhesion selection among non-adherent cells. The non-adherent PBMC were represented mostly by neutrophils and lymphocytes. In one case eosinophilia was observed. Characterization of heterospheroids confirmed the penetration of immune cells among the neoplastic population.

Conclusion
This heterospheroid model may hold the potential for advancing our understanding of macrophage involvement within the TIME in canine tumors. It allows exploration into the interactions of other immune cells in the absence of macrophages, contributing to deeper insights into their roles.

ORAL ABSTRACTS

Veterinary Pathology | Thursday 29th August 2024, 12.00-12.12

THE SKIN I LIVE IN. THE INTRACELLULAR LIFESTYLE OF P. DESTRUCTANS DURING WHITE-NOSE SYNDROME OF BATS

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Background
Millions of hibernating bats across North America have died from white-nose syndrome (WNS), an emerging disease caused by the psychrophilic (cold-loving) fungus, Pseudogymnoascus destructans (Pd), that invades their skin. The little brown bat (Myotis lucifugus) is a highly susceptible species. Mechanisms of Pd invasion and colonization of bat epidermis remain obscure.

Materials & Methods
We used electron microscopy (EM) to characterize the early stages of WNS in little brown bats. Then, we generated an immortalized little brown bat keratinocyte cell line and modeled the two phases of hibernation and Pd infection (torpor and arousal). We conducted RNA sequencing and EM to identify key features of the in vitro infection, followed by hypothesis testing experiments measuring cell death (LDH), apoptosis (caspase 3/7), and fungal invasion into bat keratinocytes. Finally, we inhibited DHN-melanin synthesis in Pd to assess its contribution to pathogenesis, and screened for polysaccharide-based virulence factors using glycomics.

Results
Guided by our in vivo observations, we uncovered an intracellular lifestyle of Pd, which inhibits apoptosis of keratinocytes and spreads through the cells by two epidermal growth factor receptor (EGFR)-dependent mechanisms: active penetration during 12°C incubation (torpor-like) and induced endocytosis during 37°C incubation (arousal-like). Melanin of endocytosed Pd blocks endolysosomal maturation, facilitating Pd survival and germination after return to torpor-like temperature. Finally, we identified a rhamnomannan highly expressed by Pd that can be involved in fungal virulence.

Conclusion
Our results unveil critical mechanisms of WNS pathogenesis and imply that Pd can adapt its invasive strategy to the host hibernation status.

ORAL ABSTRACTS

Veterinary Pathology | Thursday 29th August 2024, 12.12-12.24

DETECTION OF HIGHLY PATHOGENIC AVIAN INFLUENZA H5N1 IN MARINE MAMMALS FROM SOUTH AMERICA

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Background
Since its emergence in China in 1996, high pathogenicity avian influenza (HPAI), caused by H5N1, has evolved into a global panzootic. In addition to birds and poultry, H5N1 viruses have spread to wild and domestic mammals, as well as humans. Since 2021, unprecedented deaths in South American wild birds, poultry, and marine mammals have been reported.

Materials & Methods
Formalin-fixed, paraffin-embedded tissue (FFPE) samples (central nervous system (CNS), lung, pulmonary lymph node, heart, spleen, intestine, kidney, liver and placenta, upon availability in each animal) from 11 marine mammals found dead in coastal areas of South America in 2023 were collected for molecular diagnosis, histopathology, and immunohistochemistry (IHC).

Results
Histologically, lesions that could be attributable to HPAI were more frequently detected in the CNS and after correlation with IHC results, which typically consisted in neuronal and glial necrosis, gliosis, vasculitis, hemorrhages, spongiosis and lymphoplasmacytic and neutrophilic meningoencephalitis with perivascular cuffing. Avian Influenza Virus (AIV) -positive immunostaining was mostly detected in neurons, glial cells and ependymal cells. However, a positive correlation between RT-qPCR and IHC assessment were not found in all the animals, since in some cases, the infection by AIV was only confirmed by molecular methods.

Conclusion
The neurotropism of these H5N1 viruses in marine mammals was confirmed since the virus were systematically detected in the CNS of all evaluated animals by PCR. This was also supported by the presence of microscopic lesions and the presence of abundant AIV antigen by IHC in most of the analyzed specimens.

ORAL ABSTRACTS

Veterinary Pathology | Thursday 29th August 2024, 12.24-12.36

HIGHLY METASTATIC MAMMARY CARCINOMAS IN TWO PATAGONIAN SEA LIONS

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Background
Several tumours have been reported in pinnipeds, showing a higher prevalence in Californian sea lions. However, only few reports are available in Patagonian sea lions. Thus, this case report describes two cases of highly metastatic mammary carcinomas in this species.

Materials & Methods
Two female captive Patagonian sea lions of 13 and 25 years of age were humanly euthanized after showing inappetence, emaciation, locomotory difficulties or respiratory distress non-responsive to treatment. In the younger animal, abdominal echography revealed multiple masses in the caudal mammary tissue while CT-scan detected multiple masses in lungs and thoracic vertebras. A complete necropsy was performed and samples of the main organs were collected for histopathological evaluation.

Results
Macroscopically, multiple flat to round masses ranging from 2 to 15 cm with a whitish-brownish colour and a firm consistency were detected infiltrating the left caudal mammary glands in both animals. Multifocal nodules with similar characteristics were also observed in lungs, pleura, pericardium, liver, pancreas, adrenal glands, ovary, thoracic vertebra, brain and mammary, mandibular, prescapular, axillary, inguinal and renal lymph nodes. Histologically, all the above-mentioned masses were composed of moderately pleomorphic neoplastic epithelial cells disposed in different patterns: sieve-like arrangement (cribriform carcinoma), tubules (tubular-papillary carcinoma) or small islands with a necrotic centre (comedocarcinoma). Multifocally, the neoplastic epithelial cells contained intracytoplasmic small vacuoles (lipid-rich carcinoma).

Conclusion
Based on these findings, the tumours were classified as mammary carcinomas with multiple metastases. This is the first report of a highly metastatic mammary carcinoma in Patagonian sea lions.

ORAL ABSTRACTS

Veterinary Pathology | Thursday 29th August 2024, 12.36-12.48

PATHOLOGIES AND CAUSES OF DEATH IN STRANDED CETACEANS IN THE CANARY ISLANDS (2013-2018)

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Background

Canary Islands is a geographical spot within the North-East Atlantic ocean with a high cetacean biodiversity. Monitoring the Health Status of the different marine mammal species, through the anatomic pathology, is a key point for conservation purposes as well as keeping the balance with the anthropogenic activities.

Materials & Methods

The anatomic patholoy reports of stranded cases from 2013 to 2018 represented the principal source of information.
Necropsies, categorization, and tissue histologic interpretation of stranded carcasses were conducted following internationally accepted guidelines as well as recapitulating stablished methods from homologous antecessor studies

Results

The most probable cause of death (CD) was determined in 194/224 animals (86.6%). Anthropogenic related activity was responsible for 12.9% deaths of the stranded cetaceans while pathologies of natural origin supposed 73.6%. Within natural pathologies, infectious (e.g., morbillivirus, herpesvirus, Brucella sp, Staphylococcus aureus, Erysipelothrix rhusiopathiae, Photobacterium damselae, Clostridium perfringens...) and parastic (e.g., Nasitrema spp., Crassicauda...) events accounted for 53% and 38% of total cases, respectively. Concerning anthropic origin pathologic entities, interaction with fisheries activities was described in 17/224 (7.6%) cases, vessel collisions in 9/22 (4%), and foreign body-associated pathology in 3/224 (1.34%) animals.

Conclusion

Taking the above into consideration, this retrospective study reaffirms the trends exposed by predecessor long-term studies and significantly expands the insights of cetacean pathology, adding new descriptions of pathologic conditions in a wide range of different cetacean species.

ORAL ABSTRACTS

Veterinary Pathology | Thursday 29th August 2024, 12.48-13.00

MORTALITY IN ENDANGERED MAUI DOLPHINS

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Background
New Zealand's endemic Māui dolphins are critically endangered, with an estimated population of less than 60 (>12 m.o.). Accidental capture in fishing nets (bycatch) is considered the biggest threat to species survival, with extensive mitigation effort directed at reducing bycatch mortality. Recently, infectious disease has been suggested as an additional threat, and the small population size introduces the possibility of immunosuppression due to a 'genetic bottleneck'.

Materials & Methods
We reviewed records for 54 Māui dolphin mortalities recorded in the New Zealand cetacean stranding database from 1921-2024. From 1997 species management policy included routine recovery and necropsy of carcasses (n=21); we assigned each of these dolphins a cause of death diagnosis based on gross and histological lesions.

Results
Of the 54 total mortalities, 5 (9%) were known bycatch deaths and 28 (52%) were not investigated, including 5 carcasses post-1997 that were not recovered for necropsy. Causes of death for the 21 dolphins with necropsy data were: infectious disease (n=7 (13%)); shark predation (n=3 (6%)); probable bycatch (n=1 (2%)); chronic emaciation/parasitism (n=1 (2%)) and undetermined (n=10 (9%). Of the undetermined cases, 9 were too decomposed for diagnosis and 1 had no diagnostic lesions. Infectious diseases included toxoplasmosis (n=3), brucellosis (n=3) and aspergillosis (n=1).

Conclusion
This study highlights the difficulty of determining a definitive diagnosis in beachcast Māui dolphins and suggests that infectious disease could be a significant factor in population survival. Future work should include improving carcass recovery and investigating the role of immune function in this critically endangered population.

ORAL ABSTRACTS

Veterinary Pathology | Thursday 29th August 2024, 14.15-14.27

EXPLORING NEW DIAGNOSTIC MARKERS IN FELINE NASAL LYMPHOMA

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Background
Nasal lymphoma (FNL) is the most common nasal cavity tumor in cats. Contrastingly, feline lymphoplasmacytic rhinitis (FLPCR) is a progressive nasal disease with cycles of lymphoplasmacytic inflammation, tissue damage and repair. Not only are clinical signs similar between both entities, findings in inflammatory mass-like lesions overlap with those of FNL, and lymphoid inflammatory infiltrates are commonly seen in and around nasal lymphomas. Therefore, new diagnostic or prognostic tools are needed in clinical practice.

Materials & Methods
Cases of chronic nasal disease (n=80) diagnosed via nasal biopsy, between 2017 and 2023 at the Veterinary Teaching Hospital, University of Lisbon were divided into two groups (FNL (n=19) and FLPCR (n=61)) based on cell morphology and histopathological features. Immunohistochemistry was used to detect expression of CD3, Pax5, BCL-2, STAT5, MLH1, MSH2 and PCNA, and to characterize inflammatory and/or neoplastic lymphoid populations in both groups.

Results
In lymphomas, neoplastic lymphocytes were predominantly Pax5+, moderately to strongly STAT5+, MLH1+, MSH2+ and PCNA+, and occasionally but strongly BCL-2+. STAT5 expression in these cells was almost exclusively cytoplasmic. In both lymphoma and rhinitis cases, inflammatory lymphocytes were variably and weakly BCL2+, MLH1+, MSH2+ and PCNA+, and often weakly STAT5+. STAT5 expression in these cells was exclusively nuclear. Both CD3+ and Pax5+ cells were seen in similar numbers in rhinitis cases, while inflammatory lymphocytes were predominately CD3+ in lymphoma cases.

Conclusion
Our results suggest that STAT5, MLH1, MSH2 and PCNA show potential as diagnostic markers in differentiating lymphoma from lymphoplasmacytic rhinitis, justifying further study.

ORAL ABSTRACTS

Veterinary Pathology | Thursday 29th August 2024, 14.27-14.39

EVIDENCE OF RUSTRELA VIRUS-ASSOCIATED FELINE STAGGERING DISEASE IN SWEDEN SINCE THE 1970S

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Background
Staggering disease (SD) is a severe neurological disease regularly reported in Swedish cats since the 1970s. The aetiology of feline SD has been debated, but novel rustrela virus (RusV) was suggested as the causative agent in cases of SD in Sweden, dating from 2017 onwards. However, if RusV was also associated with earlier cases of SD in Swedish cats remained unknown. We investigated presence of RusV in historical cases of feline SD in Sweden, dating back to the 1970s, and characterized associated morphological inflammatory changes.

Materials & Methods
Formalin-fixed, paraffin-embedded brain and spinal cord, archived at the Swedish University of Agricultural Sciences, from 14 cats with plausible SD and five non-encephalitic control cats were investigated for presence of RusV antigen and RNA using immunohistochemistry and reverse transcription-quantitative PCR (RT-qPCR), respectively. Borna disease virus (BoDV)-1 RNA was investigated in plausible SD cats using RT-qPCR.

Results
Thirteen cats with plausible SD showed lymphohistiocytic infiltrates morphologically compatible with SD. The cerebrum and brainstem showed the most severe inflammatory changes. All 13 cats were RusV-positive by immunohistochemistry and 12 of them also by RT-qPCR. All 13 cats were negative for BoDV-1. Immunolabeling was mainly observed in morphologically intact neurons, typically unassociated with inflammatory infiltrates. One cat, morphologically untypical of SD, and all control cats tested negative for RusV by RT-qPCR, and showed negative or uncertain immunolabeling by immunohistochemistry.

Conclusion
We show that RusV has infected Swedish cats as far back as the 1970s, further strengthening RusV as the causative agent of feline SD.

ORAL ABSTRACTS

Veterinary Pathology | Thursday 29th August 2024, 14.39-14.51

KEY MILESTONES FOR THE DIAGNOSTIC OF EPIZOOTIC HEMORRHAGIC DISEASE IN CALVES

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Background

Epizootic haemorrhagic disease (EHD) is a non-contagious orbiviral illness, transmitted by hematophagous *Culicoides* midges, affecting wild and domestic ruminants. Recent outbreaks in Spain caused by serotype 8 have induced acute and severe disease in both young and adult cattle. The aim of this work is to describe the EHD pathology observed in calves.

Materials & Methods

Clinical signs and gross pathology were studied in animals from affected farms. Three beef calves were submitted for full pathologic examination. Spleen samples were studied by RT-PCR to confirm aetiology.

Results

Animals from affected farms and the three submitted calves were 4-7 months-old and presented with dyspnoea, fever, sero-mucous discharge and inappetence, unresponsive to non-steroidal anti-inflammatory drugs. Occasionally, calves showed locomotor disturbances associated with laminitis and coronitis. In the majority of cases, death occurred after 24-48h. Grossly, conspicuous widespread haemorrhages in the conjunctivae, subcutaneous tissue and many internal organs were evident. Remarkably, lungs presented severe alveolar oedema with large blood-clots occluding the tracheal and bronchial lumen. Erosions and ulcers in the palate, oesophagus and abomasum were commonly found. Fibrin microthrombi within small-calibre vessels, severe tissue haemorrhages and absence of inflammatory changes were microscopic key milestones. The three submitted calves tested positive for EHD virus.

Conclusion

Calves are highly susceptible to newly circulating EHD virus serotype 8, something not described previously. Pathology is similar to that reported in adult cattle and wild ruminants. In calves, a differential diagnosis with other common cattle diseases such as bovine viral diarrhoea or infectious bovine rhinotracheitis is needed.

ORAL ABSTRACTS

Veterinary Pathology | Thursday 29th August 2024, 14.51-15.03

BONE PATHOLOGY IN COMMERCIAL PIGS: TIME TO DEAL WITH THE ELEPHANT IN THE ROOM?

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Background
While swine veterinarians and pathologists are seasoned to deal with the numerous infectious diseases and pathogens that affect pigs, non-infectious diseases in general, and bone pathology in particular, can be challenging to recognize and diagnose. The latter is especially relevant when the main clinical signs and lesions do not involve the locomotor system, or when the cause of locomotor clinical signs is obvious, such as in infectious arthritis, and further investigation of bones is not pursued. Thus, the objective of this case series is to present a varied selection of representative bone pathology cases in commercial pigs, including steps from sample procurement to description of lesions and ancillary tests results interpretation.

Materials & Methods
A series of porcine diagnostic cases including pigs of different ages and clinical presentations, submitted to the Veterinary Diagnostic Laboratory of the University of Minnesota, were included in this report.
Diagnostic investigations encompassed ante-mortem observation of clinical signs and post-mortem macroscopic and histopathologic lesion assessment, bone mineral profiling, vitamin analysis and/or trace mineral analysis.

Results
Various pathologic conditions of bones, including growth plate dysplasia, osteochondrosis, bone abscesses and pathologic fractures, among others, were described and correlated with ancillary testing results.
Additionally, the pathophysiology of bone metabolism, including factors specific to farmed pigs, were reviewed.

Conclusion
Bone pathology in commercial pigs is common and underdiagnosed. By recognizing and becoming familiar to bone issues, pathologists play a fundamental role at maintaining the health and welfare of pigs.

ORAL ABSTRACTS

Veterinary Pathology | Thursday 29th August 2024, 15.03-15.15

FROM PIXELS TO PIGLETS: UTILIZING DIGITAL IMAGE ANALYSIS AND DEEP LEARNING TO COMBAT PORCINE DIARRHEA

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Background
Diarrhea is a highly prevalent clinical manifestation in pigs and a major cause of antibiotic treatments. The diseases causing diarrhea are etiologically complex, and highly related to age, with a few pathogens causing distinct lesions, while the majority of cases display only subtle macroscopic and histologic changes. The pig population is divided into susceptible and resilient genotypes, which is particularly well described for enterotoxigenic E. coli infections. Histological evaluation of intestinal tissue is traditionally a qualitative assessment, prone to subjectivity and inter-observer variability. In order to unravel the potential differences between resilient and susceptible pigs, objective and quantifiable measures are needed. Therefore, we investigated the utility of digital image analysis and deep learning models to evaluate porcine intestinal pathology.

Materials & Methods
Jejunum samples from 145 pigs with and without diarrhea, from 5 age groups (4, 14, 25, 49, and 67 days) were collected. Sections were HE stained and scanned for digital image analysis. A 3-step deep-learning model for separation of intestinal wall layers was developed using the software QuPath, and applied to all samples followed by automated cell detection.

Results
Generally, a satisfactory distinction of tissue layers for most samples was achieved, with manual correction needed for samples with intestinal content residue. Neonatal piglets with vacuolated epithelium required evaluation by a separately trained model. By applying the model, quantitative measures including area proportions and cellular densities were obtained.

Conclusion
Training of deep-learning models provides a potentially strong tool in generating quantifiable measures for evaluation of porcine intestinal pathology.

ORAL ABSTRACTS

Veterinary Pathology | Thursday 29th August 2024, 15.15-15.27

HEDGEHOG ARTERIVIRUS-ASSOCIATED ENCEPHALITIS IN CAPTIVE EUROPEAN HEDGEHOGS (ERINACEUS EUROPAEUS) IN WILDLIFE REHABILITATION CENTRES IN ENGLAND

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Background
Multiple incidents of fatal encephalitis have emerged in captive European hedgehogs (Erinaceus europaeus) in wildlife rescue centres in England. Hedgehog arterivirus (HhAV-1) has been detected by molecular tests but the causal relationship between the virus and the observed central nervous system (CNS) lesions has not been investigated.

Materials & Methods
Necropsy specimens from hedgehogs with neurological signs or histological lesions of meningoencephalitis (n=8) and a control group of hedgehogs found dead in the wild due to predation/trauma (n=13) were subjected to real-time RT-PCR, histological examination and in situ hybridisation (ISH) for HhAV-1 RNA using RNAScope®.

Results
All neurologic hedgehogs were RT-PCR positive with cycle threshold (Ct) ranging 19.13 to 24.43. HhAV-1 RNA was detected by ISH in various tissues of these hedgehogs, including the CNS and lymphoid tissues, with a tropism for monocyte-macrophages and vascular endothelial cells. Despite mild meningoencephalomyelitis, viral RNA was abundant and widely distributed in the CNS, with viral RNA typically co-localised within areas of gliosis and inflammation. Viral RNA was also present in areas of lymphoid depletion. In control hedgehogs, 8 of 13 were RT-PCR positive but with lower viral loads (Ct 29.8 to 36.2). No CNS lesions or ISH labelling was observed in a subset of RT-PCR positive control animals.

Conclusion
Preliminary findings suggest HhAV-1 CNS infection contributes to neuropathology in captive hedgehogs. While the disease pathogenesis and epidemiology remain unclear, HhAV-1 infection should be considered a differential diagnosis in hedgehogs with neurological signs or increased mortality.

ORAL ABSTRACTS

Veterinary Pathology | Thursday 29th August 2024, 15.27-15.39

COMBINING STEREOLOGY AND IMAGE ANALYSIS FOR ACCURATE AND FAST QUANTIFICATION IN EXPERIMENTAL PATHOLOGY

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Background
Though stereology is the gold standard in quantitative pathology, it's seen as complex, time-consuming, and demanding specialized equipment. Image analysis is trending due to its speed and reproducibility. Yet, precision and accuracy differ, and inferring 3D from 2D data alone can be misleading.
Our aim is to merge both methodologies, integrating stereology's sampling principles with the image analysis capability to swiftly process a large number of images, and propose a new approach as accurate as stereology and as fast as image analysis for volume and ratio estimations.

Materials & Methods
Five mice from a metastatic lung melanoma project were studied. Each lung was sectioned at uniform constant intervals, yielding 8 sections/organ. Lung volumes, metastasis volumes, and the metastasis/lung ratio were measured using the Cavalieri principle (Volume=Area x Thickness). Stereology used the Visiopharm stereology software, while image analysis used Qupath.

Results
Regarding lung volumes, metastasis volumes, and metastasis/lung ratios no statistical differences were identified between the 2 methods ($p>0,05$), with a very strong correlation coefficient ($r=0,9$; $p=0.083$).
When comparing metastasis/lung ratios between individual slides in the same mice, some outliers were found across different animals, and the coefficient of variation was always above 10% and reached more than 40%, indicating moderate to strong heterogeneity.

Conclusion
Our findings demonstrate that on volume estimations, integrating the sampling principles of stereology with the quantification methods of image analysis yields highly accurate volume estimations comparable to stereology. This study also confirms the inadequacy of relying solely on a single 2D section to accurately represent a 3D lesion.

ORAL ABSTRACTS

Veterinary Pathology | Thursday 29th August 2024, 16.15-16.27

EFFECT OF THE ORAL ADMINISTRATION OF HEAT-KILLED MYCOBACTERIUM MANRESENSIS ON INTESTINAL HEALTH AND IMMUNOLOGY PARAMETERS OF WEANED PIGLETS

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Background
Ban on antibiotics as growth promoters has led to the search for nutritional alternatives in the swine industry. The present study examines the potential of supplementing the diet of piglets with heat-killed Mycobacterium manresensis (hkMn), a safe food supplement approved by EFSA to improve intestinal health and prevent disease in adults.

Materials & Methods
Twenty 4-week-old piglets, divided into four experimental groups, were subjected to control and treated diets (supplemented with 10, 50 and 100 ppm of hkMn, respectively) for 70 days. Along the study, whole blood, serum and saliva samples were collected to determine lymphocyte subpopulations and biomarkers of interest. At the end of the study, the animals were euthanised and small intestine samples were collected to perform histopathology, histomorphometry and immunohistochemical studies.

Results
Histomorphometry showed a significant increase in jejunal villus height and crypt depth in treated groups. A decrease in CD8β+ and CD4+FoxP3+ subpopulations as well as an increase in γδ T cells was detected by flow cytometry in PBMCs of treated groups, together with an increase in the frequency of FoxP3+ cells in the small intestine, mainly in 50 ppm group. Interestingly, all treated groups displayed significantly higher saliva levels of adenosine deaminase (ADA) compared with control animals at the end of the study.

Conclusion
Supplementation with hkMn may be associated with improved intestinal absorptive capacity in the jejunum, and enhanced cellular immunity through the recruitment of regulatory T lymphocytes (FoxP3$^+$) at the small intestine as well as higher levels of ADA in saliva.

ORAL ABSTRACTS

Veterinary Pathology | Thursday 29th August 2024, 16.27-16.39

LIVER LESIONS IN SHEEP VACCINATED AGAINST F. HEPATICA WITH AND WITHOUT NALTREXONE AS IMMUNE MODULATOR

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Background
Fasciola hepatica causes important economical losses in the livestock industry and there are no commercial vaccines for this parasite. Naltrexone has been used successfully as immune modulator in vaccines against F. hepatica and Toxoplasma gondii in mice. However, it has not been tested in sheep.

Materials & Methods
Fourty-two 6-month-old sheep were divided into 6 groups (n=7) for the vaccine trial. Two peptides of FhCL1 were used as antigens. Montanide 61VG was used as adjuvant with naltrexone (group 1) or without naltrexone (group 2) and poly-IC with naltrexone (group 3) or without naltrexone (group 4). Groups 5 and 6 were used as infected control and uninfected control, respectively. Animals were immunised twice 4 weeks apart, orally infected with 150 metacercariae of F. hepatica and sacrificed 16 weeks post-infection for evaluation of fluke burden and hepatic lesions. Grossly hepatic fibrosis and scars were scored as 1 (mild), 2 (moderate), 3 (severe) and 4 (very severe).

Results
Mean fluke burdens were 53.7±17.0, 62.6±15.0, 52.4±8.6, 58.6±13.0 and 54.6±13.7 for the four vaccinated groups and the infected control group, respectively. Score for hepatic fibrosis 2.1±0.8, 1.9±1.2, 2.4±1.4, 2.0±1.0 and 2.7±0.8 for the vaccinated and infected control groups, respectively. Statistical analysis revealed no significant differences between vaccinated and infected control group.

Conclusion
The use of naltrexone did not induce protection in terms of fluke burdens and gross hepatic lesions in any of the four vaccine candidates tested in the present study.

ORAL ABSTRACTS

Veterinary Pathology | Thursday 29th August 2024, 16.39-16.51

EFFECTS OF VACCINATION ON THE IMMUNOHISTOCHEMICAL EXPRESSION OF IFN-Y AND MACROPHAGE POLARIZATION IN CALVES EXPERIMENTALLY INFECTED WITH PARATUBERCULOSIS

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Background
Silirum® vaccination provides strong peripheral Th1 immunity in calves experimentally infected with *Mycobacterium avium* subsp. *paratuberculosis* (Map), with a reduction of the intestinal lesions. However, the precise immunological response at the infection site remains unknown. The aim of this study was to evaluate the effect of Silirum® vaccination on the immunohistochemical expression of IFN-γ, iNOS and CD204 in the intestine of calves experimentally infected with Map.

Materials & Methods
Holstein claves were administered with a subcutaneous injection of Silirum® and orally challenged with Map at 60 days post-vaccination. Samples from the injection-site granuloma, intestine and associated lymph nodes were collected. Intestinal lesions were classified as focal, multifocal, and diffuse. The distribution of labeled cells for IFN-y was assessed by differential count. The staining intensity of the macrophages forming the lesions for iNOS and CD204 was compared using a H-score.

Results
Calves vaccinated with Silirum® showing focal lesions at the Peyer's patches, associated with latent infection, had higher H-score for iNOS within the granulomas. These animals showed greater numbers of lymphocytes labeled for IFN-y at the vaccine-associated subcutaneous granuloma. Calves with progressive multifocal forms (regardless of the vaccination status) showed strong H-score for CD204 in the granulomas, with upregulated expression of IFN-y at the lamina propria.

Conclusion
Parenteral vaccination with Silirum® is associated with enhanced Th1 response at the infection site, with M1-polarization (iNOS+) of the granulomas. Progression of paratuberculosis, in both vaccinated and unvaccinated cattle, is associated with a M2-polarization (CD204+), despite elevated expression of IFN-y.

ORAL ABSTRACTS

Veterinary Pathology | Thursday 29th August 2024, 16.51-17.03

EARLY PATHOGENESIS AND INNATE IMMUNE RESPONSES AGAINST TWO STRAINS OF PORCINE EPIDEMIC DIARRHOEA VIRUS IN NEWBORN AND WEANED PIGLETS

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Background
This study investigated early pathogenic events including innate immune responses of newborn and weaned pigs infected with S-INDEL or non S-INDEL strains of porcine epidemic diarrhoea virus (PEDV).

Materials & Methods
After orogastric viral inoculation in 1-week-old (n=8, 4/strain) and 1-month-old (n=8, 4/strain) piglets, body weight, temperature and clinical signs were monitored for 48 (hpi). PEDV RNA levels were assessed in rectal swabs at 0 and 48 hpi. Pathological investigations and viral load from jejunal content and intestinal mucosa were evaluated at 48 hpi. Finally, a microfluidic RT-qPCR (Fluidigm) assay was used to quantify transcripts of innate immune-related genes (antiviral and inflammatory) at 48 hpi using Laser Capture Microdissection (LCM)-derived jejunal samples.

Results
At 48 hpi, newborn piglets showed severe symptoms, while weaned piglets were mostly asymptomatic. However, clinical signs and lesions were similar in newborns regardless of the PEDV strain. Both strains were equally found in the small intestine by immunohistochemistry and similar viral loads in intestine and faeces. Moreover, villous atrophy and fusion induced by both strains were consistent across age-groups. Weaned piglets generally had higher expression of antiviral (type I and III interferon) and pro-inflammatory-related genes compared to newborns, with the non S-INDEL strain inducing greater expression of these genes in one-week-old piglets.

Conclusion
Overall, PEDV-induced intestinal damage, replication, and excretion were similar independently of the viral strain or piglets' age. Differences in clinical sign severity between ages may be partially due stronger antiviral and pro-inflammatory response in weaned piglets.

ORAL ABSTRACTS

Veterinary Pathology | Thursday 29th August 2024, 17.03-17.15

ADVERSE REACTIONS AFTER VACCINATION IN SHEEP: A REVIEW OF EXTRINSIC EVENTS OVER THE LAST 30 YEARS

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Background

Ruminants usually receive several vaccines over their lifespan. In addition to the well-known post-injection granulomas, side effects may occur due to manufacturing defects or incorrect application, which must be distinguished for fast notification and prevention. The aim of this presentation is to review different extrinsic adverse reactions following the application of commercial vaccines in sheep.

Materials & Methods

A retrospective review of cases over the last 30 years at the University of Zaragoza, identified four different extrinsic adverse reactions after vaccination in sheep. The clinicopathological presentation for each situation was reviewed.

Results

Adverse events were: 1) Clostridial growth of vaccine-containing clostridia at injection site due to contamination or lack of inactivation. There was a high incidence and mortality associated with subcutaneous oedema in multiple farms after vaccination with a specific enterotoxaemia bacterin. In all animals, focal subcutaneous oedema in the flank extending to ventral areas was observed. A single, approximately 1-millimetre necrotic foci was always found within the flank and clostridial vegetative forms were detected intralesionally. 2) Contamination of a live attenuated orf vaccine with a bovine viral diarrhoea virus 2. A highly- prolific flock had 72.7% of abortions, stillbirths and neurological malformations in a single lambing season. 3) Bacterial contamination at the injection site due to improper asepsis, linked to extensive necrotizing cellulitis in lambs. 4) Accidental routes for vaccine administration: intrathoracic injection.

Conclusion

Various extrinsic vaccine adverse reactions can be detected in sheep. Knowledge on their pathologic features will aid in reaching a quicker diagnosis and response.

ORAL ABSTRACTS

Veterinary Pathology | Thursday 29th August 2024, 17.15-17.27

DIGITAL HISTOPATHOLOGY ANALYSIS OF LYMPHOID ORGANS FROM GOATS EXPERIMENTALLY INFECTED WITH HIGHLY AND MILDLY VIRULENT PPRV STRAINS

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Background

Peste des Petits Ruminants (PPR) is a reportable disease caused by PPR Virus (PPRV). Histological lesions and immunohistochemistry (IHC) staining are traditionally assessed semiquantitatively. The aim of this study is to perform a quantitative digital histopathological analysis in PPRV-infected caprine lymphoid organs, which may increase the analysis accuracy and its objectivity.

Materials & Methods

Three groups of adult Saanen goats (n=6 each) were intranasally inoculated with: 1) Morocco 2008 (MA08) (highly virulent PPRV strain); 2) Côte d'Ivoire 1989 (IC89) (mildly virulent PPRV strain) and 3) mock inoculated. Three goats per group were euthanized and necropsied at 3 and 6 days postinfection (dpi). The main lymphoid organs were fixed in formalin, and IHC for PAX-5, CD3, Iba-1 and the N-Protein of the PPRV were performed. Whole slides were scanned and analysed using an artificial intelligence-powered software (Visiopharm).

Results

The maximum IHC PPRV positivity density occurred at 6 dpi, with much higher density in MA08-infected than in IC89-infected goat tissues, particularly within lymphoid follicles, tonsillar epithelium and ileal subepithelial dome. MA08-infected goats suffered a reduction of PAX-5 density in all lymphoid organs, a reduction of CD3 density within lymphoid follicles of lymph nodes and splenic white pulp, and an increase of Iba-1 density in the lymph nodes and Peyer's Patches.

Conclusion

The digital analysis demonstrated higher tissue viral densities in MA08 than in IC89-inoculated goats, accompanied by a lymphocytic depletion and an infiltration of Iba-1 positive cells. PPRV may use both tonsils and Peyer's Patches as portal of entrance.

ORAL ABSTRACTS

Veterinary Pathology | Thursday 29th August 2024, 17.27-17.39

QUANTITATIVE AND QUALITATIVE ASSESSMENT OF HISTOLOGIC LESIONS IN HONEY BEES (APIS MELLIFERA) INFECTED WITH CHRONIC BEE PARALYSIS VIRUS

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Background
Chronic bee paralysis virus (CBPV) is an emerging threat to honey bees (Apis mellifera), causing clinical signs such as trembling and flightlessness and can lead to colony collapse due to high mortality. Previous work has demonstrated the presence of the virus in the brain, but there is no description of the histologic lesions resulting from infection, or their temporal progression.

Materials & Methods
Adult honey bees were experimentally infected with CBPV via coelomic injection and sampled at one, two, and three days post-infection (dpi), with control honey bees receiving saline injections. At 3 dpi, infected bees were further categorized based on severity of clinical signs into groups showing early partial paralysis (3EPP), late partial paralysis (3LPP), paralysis (3P) and deceased (3D). Histopathological sections of bees were examined using optical microscopy with quantitative assessment of spongiosis using QuPath.

Results
Severe lesions were observed in the central nervous system from 3 dpi, including spongiosis, neuronal necrosis, and increased hemocytes within pericerebral sinuses. 3EPP bees showed milder lesions compared to other groups. Additionally, basophilic stippling was prevalent in flight muscles, alongside muscle degeneration in some cases. In the small intestine, basophilic, round, cytoplasmic globules were found in bees classified as 3D and 3LPP. Quantitative analysis showed an increase in percentage spongiosis in the brain and ganglia of 3LPP, 3P and 3D bees compared to control and 3EPP bees.

Conclusion
CBPV resulted in spongiosis, neuronal necrosis and increased hemocytes in the central nervous system of honey bees, with other changes observed in the flight muscles and small intestine.

ORAL ABSTRACTS

Veterinary Pathology | Thursday 29th August 2024, 17.00-17.12

SLC25A12-RELATED INHERITED FORM OF PORENCEPHALY IN LIMOUSIN CALVES

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Background
Porencephaly is typically associated with teratogenic viruses in ruminants. However, rare heritable forms have been described in domestic ruminants. Thirteen clinically and pathologically similar cases of porencephaly in related purebred Limousin calves suggested a monogenic recessive inherited cause.

Materials & Methods
A thorough diagnostic investigation was performed, including pathological examination of calves, PCR and serology for specific teratogenic viruses, and whole-genome sequencing of three affected calves and their sire.

Results
Affected calves presented in early life with blindness and stupor from birth and were unable to suckle without assistance. Cases came from several herds located in the north of England and Wales. Some herds had only one affected calf, while others had multiple cases over successive calving seasons. Brain examination revealed bilaterally symmetrical cavities in the cerebral cortex (porencephaly). Some cases also showed microscopic changes in the cerebellar cortex indicating ongoing neuronal and axonal degeneration. Bluetongue, Schmallenberg and bovine viral diarrhoea viruses, were excluded through testing. Analysis of breeding records revealed common ancestry among cases. Genetic analysis identified a rare recessive missense variant in SLC25A12, a gene known to be associated with neurodevelopmental disorders. Only affected calves were homozygous for the variant and a query of genomic data of ›5,000 animals further revealed that the variant was only observed heterozygous in related Limousin cattle.

Conclusion
While investigation of cases of porencephaly in aborted, stillborn and live-born calves should rightly focus on testing for teratogenic viruses (including bluetongue virus), the possibility of a genetic basis should also be considered.

ORAL ABSTRACTS

Veterinary Pathology | Thursday 29th August 2024, 17.12-17.24

DISTINCT SUBCELLULAR DYNAMICS OF LISTERIA MONOCYTOGENES IN MICROGLIA AND MONOCYTE-DERIVED MACROPHAGES: IMPLICATIONS FOR THE PATHOGENESIS OF NEUROLISTERIOSIS

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Background
Listeria monocytogenes (Lm) infection causes potentially fatal rhombencephalitis in both humans and ruminants. Despite its severity, the pathogenesis of neurolisteriosis, especially the specific contributions of microglia, the resident brain macrophages, and monocyte-derived macrophages (MDM), remains poorly understood.

Materials & Methods
We assessed the intracellular lifestyle of Lm in bovine MDM and microglia ex-vivo, obtained from cases of neurolisteriosis in cattle and screened with macrophage markers (P2RY12, F13A1, and IBA1), and in vitro in cultured bovine primary microglia and MDM, using immunofluorescence and confocal microscopy.

Results
Our findings reveal distinct responses to Lm infection between MDM and microglia. MDM are less permissive than microglia to Lm infection ex-vivo and in-vitro, confining the bacterium primarily to the phagolysosomal system. Conversely, a large subset of Lm in microglia enters the cytosol, replicates, and spreads to neighboring cells. Treatment with bafilomycin-A1, a V-ATPase inhibitor, significantly reduces the bacterial burden in microglia but not in MDM. In contrast, treatment with cytochalasin D, an actin polymerization inhibitor, has no effect on bacterial burden. Analysis of LAMP1 distribution reveals higher proportions of Lm-containing vacuoles decorated with LAMP1 in MDM compared to microglia with insignificant changes upon bafilomycin-A1 treatment. Correspondingly, a greater percentage of Lm is associated with lysotracker-positive acidic vacuoles in MDM compared to microglia.

Conclusion
Our data suggest that MDM control Lm infection by sequestering Lm in pre-fusional phagosomes, with a subset of bacteria confined in LAMP1+ acidified phagosomes. Conversely, microglia allow rapid bacterial escape from vacuoles, possibly providing a bacterial reservoir and promoting infection propagation in the brain.

ORAL ABSTRACTS

Veterinary Pathology | Thursday 29th August 2024, 17.24-17.36

SUSTAINED REDUCTION OF 2-OXOGLUTARATE DEHYDROGENASE COMPLEX ACTIVITY IN RAT BRAIN AFTER VENTRICULAR FIBRILLATION CARDIAC ARREST MAY CONTRIBUTE TO EXTENDED EXCITOTOXICITY

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Background

Cardiac arrest (CA) leads to global ischemia and frequently causes delayed neuronal dysfunction. Since accumulation of toxic glutamate levels is a known mechanism of excitotoxicity and neuronal damage, we hypothesized that delayed neurodegeneration may be associated with decreased activity of the glutamate metabolizing enzyme system, 2-oxoglutarate dehydrogenase complex (OGDHC).

Materials & Methods

Brains of male adult Sprague Dawley rats subjected to 6 (n=10) and 8 (n=5) minutes of ventricular fibrillation CA and extracorporeal cardiopulmonary resuscitation and sham operation (n=10) were harvested two weeks after resuscitation. One brain half was used for descriptive histological examination after HE-staining and semiquantitative assessment of OGDHC expression by immunohistochemistry. Homogenates of hippocampus and cerebral cortex of the other half were used for the quantification of OGDHC activity determined in vitro by the rate of NADH formation from 2-oxoglutarate.

Results

In the hippocampal CA1 region of all CA animals' loss of pyramidal neurons and increased glial response were detected. Hippocampal OGDHC protein expression was significantly reduced in the 6-minute group, and OGDHC activity was significantly reduced in both CA groups in relation to CA duration, compared to sham. In the cerebral cortex no lesions were detectable in HE-staining. However, OGDHC protein was significantly decreased in 8 min CA animals, which was paralleled by a decreased activity by trend (p=0.051).

Conclusion

Reduced hippocampal OGDH protein expression and activity are associated with histological damage, suggesting ongoing excitotoxicity two weeks after CA. Our data further suggest that, despite appearing histologically unaffected, cortex displays functional deficits long-term after CA.

ORAL ABSTRACTS

Veterinary Pathology | Friday 30th August 2024, 12.00-12.12

TUMOR INFILTRATING LYMPHOCYTES (TILS) VARIES IN DIFFERENT CANINE SOFT TISSUE SARCOMA (STS) TYPES.

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Background
Soft tissue sarcomas (STS) are conventionally viewed as non-immunogenic (immunologically "cold"). However, recent studies suggest some human STS may elicit an immune response thus representing potential candidates for immunotherapy. Data for canine STS are limited. To fill this gap, this study aims to assess tumor-infiltrating lymphocytes (TILs) in canine STS.

Materials & Methods
Five canine STS histotypes were retrospectively collected: 22 perivascular wall tumors (PWTs), 17 fibrosarcomas, 16 myxosarcomas, 19 liposarcomas and 18 leiomyosarcomas. Of these 87 tumors 56 were grade I, 25 grade II and 6 grade III. Cases were reviewed, graded, stained for CD3, CD20 and FoxP3 and corresponding slides were scanned. T-cells, B-cells and Tregs were quantified with QuPath software. T-cell, B-cell, Tregs, and total TILs density was expressed as number of cells in 1 mm^2. The B/T-cell ratio, and Treg proportions were calculated.

Results
Total TILs density was higher in PWTs (p=0.006) and myxosarcomas (p=0.02). PWTs had higher T-cell density (p=0.006) but lower Treg proportion (P=0.017) and myxosarcomas had a higher Treg density (p=0.02) and B/T-cell ratio (p=0.011). No association with grade was evidenced.

Conclusion
The results suggest that TILs vary in canine STS types and that PWTs and myxosarcomas may represent the most immunogenic histotypes. Myxosarcomas elicit a B-cell and Treg rich immune response; PWTs stimulate a T-cell rich and Treg poor reaction. Thus, the immune system may be involved in PWT control contributing to the less aggressive behavior of PWTs compared to other STS types.

ORAL ABSTRACTS

Veterinary Pathology | Friday 30th August 2024, 12.12-12.24

ASSOCIATION BETWEEN DOMESTIC CAT HEPADNAVIRUS AND FELINE HEPATOCELLULAR CARCINOMA

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Background
Hepatitis B virus (HBV) is a major cause of hepatocellular carcinoma (HCC) in people. The related virus, domestic cat hepadnavirus (DCH), infects cats worldwide. A pathogenic role for DCH in feline HCC has been suggested by a reported association between DCH DNA and HCC in 29 cases. Confirmation with a larger sample size is warranted.

Materials & Methods
Archived formalin-fixed paraffin-embedded feline HCC and controls (non-inflammatory/non-neoplastic, normal liver) from USA, Taiwan, and United Kingdom, were reviewed by two veterinary pathologists. DNA was extracted, tested with DCH-specific conventional PCR (cPCR) and positives were confirmed by sequencing. In cPCR-positive samples, in situ hybridization (ISH) was performed with a probe targeting DCH DNA or its mRNA transcripts (V-FeHepadnavirus), and controls (feline PPIB, housekeeping and Escherichia coli dihydrodipicolinate reductase, negative control; RNAScope).

Results
17/77 (22%) HCCs, 0/10 (0%) non-inflammatory/non-neoplastic lesions, and 0/34 (0%) histologically normal liver samples were DCH cPCR positive. Most DCH-positive HCCs were trabecular subtype with high pleomorphism and mitotic count. Eleven/17 PCR positive cases were DCH-ISH positive, with predominantly nuclear localization in tumor regions, and both cytoplasmic and intranuclear viral DNA detection in adjacent tissues. DCH signal was restricted to non-tumor regions in 3 cases.

Conclusion
We observed an association between DCH DNA detection and feline HCC. Heterogenous DCH probe localization, as seen with HBV, suggests complex virus-tumor interactions, with active viral replication in adjacent tissue, and possibly truncated, integrated genome or low viral replication in tumor clones. Our findings support a potential pathogenic role for DCH in HCC in cats.

ORAL ABSTRACTS

Veterinary Pathology | Friday 30th August 2024, 12.24-12.36

PATIENT-DERIVED EXPLANTS FROM CANINE MAMMARY GLAND CANCER USING HETEROLOGOUS PLATELET-RICH PLASMA AS CULTURE MEDIUM

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Background
Patient-derived explants (PDEs) are whole fragments of tissue that are cultured in vitro and provide a valuable model for studying disease biology and testing new therapies. Platelet-rich plasma (PRP) is obtained from homologous blood and has a high concentration of bioactive molecules released by the platelets that promote tissue healing and regeneration. This study aims to describe the generation of PDEs from canine mammary gland cancer cultured using heterologous PRP as an unconventional culture medium.

Materials & Methods
Mammary gland tumour samples obtained from four mastectomies were cut into 5 x 5 x 2 mm consecutive pieces. One specimen was placed directly in 10 % formalin (T0) while the other specimens were cultured in PRP (PS) and saline solution (SS) at 4C° for T3, T7, T14, and T21 days. FFPE sections were stained with HE and immunostained for epithelial, mesenchymal, apoptotic, and proliferative markers.

Results
HE revealed that the tumour architecture of PDEs was well conserved until T7 both in SS and PS, yet IHC revealed that PS had a better proliferative activity and viability. At T14 and T21 only PS showed a high percentage of viable tissue and retained a good preservation of antigenicity.

Conclusion
This study shows that PDEs of freshly resected canine mammary gland tumours obtained from surgery provide a high-throughput and cost-effective model that retains native tissue architecture and cell viability.

ORAL ABSTRACTS

Veterinary Pathology | Friday 30th August 2024, 12.36-12.48

HIGH-THROUGHPUT MULTI-DRUG SCREENING FOR IDENTIFYING POTENTIAL NEW THERAPIES IN MUCOSAL MELANOMA

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Background
Mucosal Melanoma (MM) is a very aggressive tumor in humans as well as in dogs, where the incidence is much higher. Recurrently mutated cancer-driver genes are shared between the two species and, basing on their molecular profile, MMs can be classified in “hot immune” and “cold immune” melanomas, the latter potentially sensitive to targeted therapy. Effective targeted therapies are lacking, and the frequent development of resistance to immunotherapy highlight the urgent need to find new effective compounds. The aim of this study was to identify effective compounds for the treatment of human and canine MM.

Materials & Methods
A high-throughput multidrug screening was performed on two human MM, five canine MM and one control canine keratinocytes cell lines. To select the chemotherapeutic and targeted compounds with the highest sensitivity, cells were treated with 224 drugs (more than half already used in standard-of-care protocols or undergoing clinical trials). Next, a follow-up combinatorial multi-drug screening was performed, testing the whole library in combination with the MDM2-inhibitor Idasanutlin. Drug-response curves were established and effective compounds were identified using the IC50 and the areas under the curves (AUCs).

Results
MDM2-inhibitors showed high efficacy in both species, especially in a metastatic cell line overexpressing MDM2. Moreover, increased efficacy was observed when Idasanutlin was combined with compounds targeting the PI3K-Akt signaling pathway.

Conclusion
Idasanutlin administered in combination with compounds targeting PI3K-Akt signaling pathway represents a potential candidate for the treatment of canine and human MM. Validation experiments to investigate this potential treatment are currently ongoing.

ORAL ABSTRACTS

Veterinary Pathology | Friday 30th August 2024, 12.48-13.00

FELINE NASAL PLANUM SQUAMOUS CELL CARCINOMA: PRELIMINARY INVESTIGATION ON EPITHELIAL TO MESENCHYMAL TRANSITION (EMT) AND FELINE PAPILLOMA VIRUS INFECTION STATUS

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Background
Papillomaviruses (PV) are known to cause a wide range of diseases in animals and humans. Among these, squamous cell carcinomas (SCC) are the most life threatening, consequences of PV infections. The presence of feline papillomavirus (FCaPV) has been recently investigated in a few head and neck SCC together with the occurrence of a partial epithelial to mesenchymal transition (EMT). In our study, we focused on one particular SCC location, the nasal planum. Accordingly, we investigated the presence of FCaPV-1, -2, -3, -4, -5 and the occurrence of the EMT process in feline nasal planum samples.

Materials & Methods
Ten fresh samples from nasal planum SCC and 6 fresh samples from normal nasal planum were used to detect FCaPV-DNA by qPCR. Sixty FFPE samples of nasal planum SCC coming from different institutions and 10 FFPE samples of normal nasal planum were retrospectively selected and histologically evaluated. Expression of E-cadherin, N-cadherin, β-catenin, vimentin, cytokeratin AE1/AE3, ZEB-1, TWIST-1 and HIF-1α was semi-quantitatively analyzed by immunohistochemistry (IHC).

Results
FCaPV-2, FCaPV-3 and FCaPV-4 DNA was detected in 7/10, 3/10, 1/10 cases, respectively. Two cases displayed FCaPV-2, -3, -4 and FCaPV-2, -3 co-infection. IHC semi-quantitative evaluation revealed lower scores for cytokeratin and E-cadherin as well as a higher scores of vimentin and N-cadherin compared to the normal tissue.

Conclusion
Results from fresh samples suggest that FCaPV-2 is the more frequently detected PV in feline nasal planum SCC. IHC evaluations are suggestive of an EMT process of the feline nasal planum SCC.

ORAL ABSTRACTS

Veterinary Pathology | Friday 30th August 2024, 13.00-13.12

78 | DIGITAL KARYOMETRY CONFIRMS INCREASED NUCLEAR SIZE AS DIAGNOSTIC TUMOR MARKER FOR CANINE UROTHELIAL CARCINOMA

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Background
Urothelial carcinoma (UC) is the most common form of bladder cancer in dogs, which accounts for 2% of all malignant tumors. Due to sampling by transurethral endoscopy, UC biopsies tend to be small, superficial and prone to squeezing artefacts which can make an accurate diagnosis difficult. Cell features which indicate malignant urothelial transformation include increased nuclear size and anisokaryosis. With the advent of digital pathology, karyometry has become feasible.

Materials & Methods
This retrospective study included digitized HE stained tissue sections of bladder and urethral UC (n=154) and non-neoplastic bladder (n=42) from 143 different dogs. The urothelium to be assessed was defined as region of interest (ROI) and the nuclear area of individual urothelial cells was measured. Digital analysis was performed with Visiopharm software (Horsholm, Denmark) utilizing deep learning (DL) classifiers and automatization for ROI definition, nuclear detection and morphometry (area, form factor, diameter, axis, perimeter). Statistical analysis was performed with RStudio (2023.12.1).

Results
Based on the size of urothelial nuclei, defined as nuclear area (µm2), the results were the following: i) malignant nuclei were significantly enlarged (median 43 µm2 vs. 29 µm2); ii) largest malignant nuclei were more than 10 times the normal size; iii) variation in nuclear size (anisokaryosis) was increased in malignant compared to non-neoplastic urothelium; iv) within UC, the expansile tumor margin had the largest nuclei compared to other tumor regions.

Conclusion
Digital pathology enables precise and automated karyometry which can be utilized to detect increased nuclear size indicative of canine urothelial carcinoma.

ORAL ABSTRACTS

Veterinary Pathology | Friday 30th August 2024, 14.15-14.27

FREQUENCY OF DETECTION OF PORCINE CIRCOVIRUSES IN RETROSPECTIVELY SELECTED CASES OF PORCINE DERMATITIS AND NEPHROPATHY SYNDROME

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Background
Porcine dermatitis and nephropathy syndrome (PDNS) is considered a type-III hypersensitivity reaction. Although there is a lack of definitive proof, porcine circovirus (PCV) 2 has been considered the main triggering antigen. The recent discovery of PCV-3 and PCV-4 and their putative association with cases of PDNS has blurred the causality link between PCVs and PDNS. This study aimed to retrospectively investigate the presence of all known PCVs in cases of PDNS.

Materials & Methods
Formalin-fixed, paraffin-embedded tissue samples previously diagnosed with acute PDNS by characteristic gross and histological lesions (period 1997-2020) were investigated for all PCVs by PCR or qPCR. Additionally, PCVs presence within tissues was determined through immunohistochemistry (IHC)/in situ hybridization (ISH) and ISH for PCV-2 and PCV-3, respectively.

Results
All cases (102/102; 100%) were PCV-2 qPCR positive (mean: $1.0 \cdot 10^7$ PCV-2 copies per mL of tissue supernatant) while 30/102 (29.4%) cases were positive to PCV-3 qPCR (mean: $2.47 \cdot 10^3$ PCV-3 copies/mL of tissue supernatant). All cases were negative for PCV-1 and PCV-4. Presence of PCV-2 by IHC/ISH was observed in 63/102 (61.8%) of the cases. By ISH, PCV-3 was found in 4 cases (tested only in those with qPCR values $>10^4$ copies/mL).

Conclusion
Obtained results point out PCV-2 as the only consistent PCV linked to PDNS, since it was detected by qPCR in all cases. Since PCVs are considered ubiquitous, presence of PCV-3 in a subset of cases (29.4%) may reflect coinfection rather than eventual causality. Viral presence within tissues was, by far, more frequent for PCV-2 than PCV-3.

ORAL ABSTRACTS

Veterinary Pathology | Friday 30th August 2024, 14.27-14.39

IDIOPATHIC INTERSTITIAL PNEUMONIAS IN FOALS FROM CALIFORNIA, 1990-2020

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Background
Idiopathic interstitial pneumonias affect young foals in California and elsewhere. Unidentified viruses, toxins, hyperthermia, certain antibiotics, and aberrant responses to Rhodococcus equi have been proposed as possible causes. This study aims to describe necropsy findings in foals with idiopathic interstitial pneumonia.

Materials & Methods
A search of the CAHFS database identified 41 necropsies of 1-12-month-old foals diagnosed with interstitial/bronchointerstitial pneumonia of undetermined etiology between 1990 and 2020.

Results
Foals had a median age of 3 months and were mostly received in summer (28/41, 68%) and spring (10/41, 24%). Fever and antibiotic treatment were reported in 19/41 (46%) and 21/41 (51%) cases, respectively. Grossly, lungs were rubbery to firm (35/39, 90%) and did not collapse (22/39, 56%). Histologically, combinations of exudative (E; hyaline membranes), proliferative (P; type II pneumocyte hyperplasia), and fibrotic (F; fibroplasia) phases predominated in the interstitium (E+P: 15/41, 37%; E+P+F: 13/41, 32%). Necrosis of bronchiolar epithelium was rare (4/41, 10%), concurrent bronchopneumonia was common (22/41, 54%), and a few foals (5/41, 12%) had pyogranulomas. Bacteria were recovered from the lungs in 21/41 (51%) cases. R. equi and E. coli were the most common isolates (9 and 7 cases each). Pneumocystis spp. cysts were observed in 8/38 (21%) cases. Equid alphaherpesviruses-1/-4, influenza A virus, Mycoplasma spp., and Salmonella spp. were not detected. Viral isolation was always negative.

Conclusion
The interstitial component predominated in these cases and represents an active, non-reparative continuum. There was no etiologic commonality, but multiple factors, including concomitant bacterial bronchopneumonia, may play a role.

ORAL ABSTRACTS

Veterinary Pathology | Friday 30th August 2024, 14.39-14.51

OVINE ICHTHYOSIS CONGENITA: DESCRIPTION AND CLINICOPATHOLOGIC EVOLUTION

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Background
Ichthyosis congenita (IC), a non-epidermolytic ichthyosis, has been described in dogs and cattle and it is associated with different gene defects. A single case of ichthyosis fetalis (epidermolytic ichthyosis) has been reported in a lamb. The aim of this work is to describe the clinicopathologic evolution of IC in ovine.

Materials & Methods
A flock of 2,000 Rasa Aragonesa sheep presented cases of progressive hyperkeratosis and scaly skin in non-wooled areas, appearing in 3-5 months old lambs (2.5% incidence). Clinicopathologic evolution was studied in an affected ewe and 4 of its lambs, born from different mating with unaffected males in two lambing seasons. Physical examination, complete blood and skin biopsies were undertaken periodically. Serum zinc levels were measured.
A genealogical study of the animals included in the study was performed.

Results
The ewe and only the two lambs from the second lambing (mated with her son) were clinically affected. Face, neck and forelimbs showed hyperkeratosis and scaly skin. Dark and seborrheic wool was also presented. Lesions extended over time to wooled areas, finally affecting the whole body surface. Microscopic lesions evolved from a moderate epidermal and follicular orthokeratotic hyperkeratosis with light eosinophilic perivascular dermatitis to a severe hyperkeratosis with diffuse lymphoplasmacytic dermatitis. Adnexal glands showed an array of dysplastic changes. Serum zinc levels were normal and affected animals were non-responsive to zinc supplementation. A possible autosomal recessive pattern of inheritance was observed.

Conclusion
To the best of the authors' knowledge, this is the first description of ovine ichthyosis congenita.

ORAL ABSTRACTS

Veterinary Pathology | Friday 30th August 2024, 14.51-15.03

ULTRASTRUCTURAL AND TRANSCRIPTOME ANALYSIS OF CEREBELLUM IN PIGLETS BORN WITH CONGENITAL TREMOR TYPE A-II, A LONGITUDINAL STUDY

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Background
Atypical porcine pestivirus (APPV) cause congenital tremor (CT) A-II in piglets, a global neurological disease. Piglets are born shaking and can suffer from starvation and immobility. Surviving pigs typically recover after 3-4 months. Little is known about the pathogenesis as the disease progress. Our objectives were to quantify ultrastructural changes in cerebellar white matter in pigs born with severe signs of CT A-II, investigate the functional effect of APPV on cerebellar cells, and observe how these aspects change as CT-affected piglets recover.

Materials & Methods
A case-control study was performed in 2021-2022 in south-eastern Norway. Pigs from three age groups (newborns, 3-weeks-old, 4-5-months-old), were sampled from herds with outbreaks of CT A-II and from a herd with healthy pigs (n=30). All CT-affected pigs were confirmed APPV-positive by RT-qPCR. Cerebellar white matter was investigated with TEM and cerebellar gene expression profiles were assessed by transcriptome analysis of pooled samples.

Results
4-5-months-old CT-affected pigs showed minimal signs of tremor. In newborns and 3-week-old CT-affected pigs, we observed decreased myelination and mild myelin disruption in cerebellar white matter. Myelination in 4-5-months-old was comparable to controls. Key genes involved in oligodendrocyte maturation were downregulated in 3-week-old CT-affected piglets, but upregulated in 4–5-months-old pigs who had recovered from CT.

Conclusion
APPV can persist in CNS in pigs clinically recovered from CT A-II. Our findings suggest that APPV-infection disrupt the maturation of oligodendrocytes, leading to delayed myelination and hypomyelination in cerebellum. Clinical recovery appears associated with compensatory upregulation of genes involved with oligodendrocyte maturation.

ORAL ABSTRACTS

Veterinary Pathology | Friday 30th August 2024, 15.03-15.15

CARPAL CONTRACTURE - A PILOT STUDY OF AN INCREASING FRONT LEG PROBLEM IN NORWEGIAN PIGS

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Background
In recent years, concerns about increasing front leg problems in grower-finishing pigs were reported in Norway, specifically pigs unable to fully extend the carpal joint when viewed from the side (carpal contracture).

Materials & Methods
Thirty-four pigs, 57-100 kg, were necropsied, 18 cases and 16 controls. A standardized necropsy protocol was created to examine bones, joints, tendons, and muscles affecting the carpus. The angle was measured, before and after cutting relevant tendons and ligaments in a predetermined order. HE slides from muscle, tendons and bones were investigated. Blood samples were analysed for calcium, magnesium, phosphorus, creatine-phosphokinase, vitamin D, vitamin E, osteocalcin and C-terminal telopeptide (CTX), the latter two biomarkers of osteoblast and osteoclast activity, respectively.

Results
The angle of the carpal joint was 151-175 degrees in affected pigs, and all affected pigs had bilateral carpal contracture. Cutting the relevant tendons and ligaments only minimally affected the carpal angle: superficial and deep digital flexors, carpal radial and ulnar flexors, and the extensor ulnaris muscle (the latter being a flexor in pigs). The contracture was caused by a tight ligament on the palmar aspect of the carpal bones. No other gross lesions were detected. Histological and blood samples did not reveal any differences between the groups.

Conclusion
The acquired carpal contracture was not secondary to other painful disease processes, and the primary cause could not be determined. The condition may be related to rapid growth, genetics, diet composition or management factors that needs to be investigated in a larger epidemiological study.

ORAL ABSTRACTS

Veterinary Pathology | Friday 30th August 2024, 15.15-15.27

BLUETONGUE IN BIGHORN SHEEP IN CENTRAL/SOUTHERN CALIFORNIA

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Background
Bluetongue virus (BTV) is an Orbivirus that causes severe disease in wild and domestic ruminants. While more than 24 serotypes have been described globally, BTV-2, 10, 11, 13, and 17 are the most common serotypes circulating within the western United States. Here we present a series of cases of bluetongue in bighorn sheep (Ovis canadensis).

Materials & Methods
Between July and October of 2023, five carcasses and one spleen sample from adult bighorn sheep located at two facilities within southern/central California, were received by the California Animal Health and Food Safety Laboratory system (CAHFS). Necropsies and BTV RT-qPCR and serotyping were performed.

Results
Five of the six animals were positive for BTV on RT-qPCR with Ct values ranging from 18.24 to 32; serotypes 11, 13, and 17 were detected. Clinical course ranged from 1 day to 3 weeks. The main gross lesions included severe pulmonary edema (4/4), endocardial hemorrhages (4/4), hemorrhage in the base of the pulmonary artery (1/4) and subcutaneous edema (1/4). Histologically, there were cardiomyocyte degeneration and necrosis (2/4) with lymphocytic myocarditis (1/4), lymphocytic myositis in the pharynx and esophagus (1/4), and aspiration pneumonia (2/4).

Conclusion
This case series demonstrates the variable gross/microscopic lesions of BTV within bighorn sheep as well as the presence of multiple serotypes of the virus causing disease in several animals within a fairly restricted geographic area.

ORAL ABSTRACTS

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PNEUMONIA IN CALIFORNIA DONKEYS

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Background
Respiratory diseases lead to considerable morbidity and mortality in equids. We retrospectively investigated pneumonia cases in donkeys with an emphasis on pathology and etiology.

Materials & Methods
Thirty-nine donkeys with pneumonia submitted to CAHFS (2001-2023) for postmortem examination were selected. The cases were categorized by morphologic diagnosis and etiology.

Results
Seasonal distribution was 31% (fall), 26% (winter), 23% (spring), and 20% (summer). The cases included neonates (n=4), juveniles (n=14), and adults (n=21). Pneumonia accounted for 17 out of 39 deaths. Extrapulmonary causes of death were gastrointestinal disease (n=6), hepatic lipidosis (n=2), and septicemia/toxemia (n=4). Bronchopneumonia (n=26), interstitial pneumonia (n=10), and embolic pneumonia (n=3) were identified. Bacterial and/or viral coinfections were diagnosed in 14/39 cases. The most common bacteria isolated in cases of bronchopneumonia were Streptococcus equi ssp. zooepidemicus (S. zooepidemicus) (9/26), Klebsiella spp. (3/26), and Escherichia coli (3/26). Real time PCR for equid herpesvirus 1/4 (EHV1/4) and influenza A virus (IAV) was performed in 19/26 cases. EHV1/4 was detected in 3/26 and IAV in 2/26. (IAV was further characterized as H3N8 in 1 case). Bacteria identified in interstitial pneumonia cases include S. zooepidemicus (2/10), Klebsiella spp. (2/10), and Escherichia coli (1/10). Viral etiology was investigated in 3/10 interstitial pneumonias; and 1/3 tested positive for IAV. In cases of embolic pneumonia bacteria and/or Aspergillus spp. were detected.

Conclusion
Bronchopneumonia and interstitial pneumonia were predominant, with S. zooepidemicus as the most common etiology. EHV1/4 and IAV were the two most prevalent viruses, warranting further investigation into their impact on donkey health.

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MULTIPLEX IMMUNOHISTOCHEMISTRY AND SUPERVISED MACHINE LEARNING FOR IMMUNE CELLS TOPOGRAPHY IN CANINE PDL1 TESTED UROTHELIAL CARCINOMA

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Background
Immune checkpoint inhibitors (ICIs) are a promising therapy in stopping tumor immune-evasion; the response depends on cancer cells and tumor immune microenvironment (TIME) interaction. This work aimed to characterize TIME and its relationship with PDL1 in canine urothelial carcinomas (UCs).

Materials & Methods
UCs were retrospectively selected and tested for PDL1 using single-antibody immunohistochemistry. Multiplex immunohistochemistry was performed with anti-CD3, -CD20, and -IBA1 antibodies, to co-localize the immune cells (ICs). ICs were quantified with supervised machine learning (QuPath) as pixel percentage in the tumor area. Based on the spatiality and density of ICs, tumors were classified in three immune phenotypes: immune-inflamed, immune-excluded, and immune-deserted.

Results
Five of 28 collected UCs (18%) were PDL1+. Twenty-two carcinomas were immune-inflamed (3 PDL1+; 19 PDL1-), 5 immune-excluded (2 PDL1+; 3 PDL1-), and 1 immune-deserted (PDL1-). Although T-cell and macrophage density was higher in PDL1+ (CD3 4.7%; IBA1 8.01%) than PDL1- cases (CD3 3.22%; IBA1 4.47%), there was no significant association between PDL1 and ICs density, nor with immune phenotype. Of the total series, the most numerous ICs were macrophages (5.1%) and T-cells (3.48%), with fewer B-cells (1.89%; $p<0.0001$), regardless of immune phenotypes. Follow-up data were available in 6 patients (4 died and 2 alive). Mean overall survival was 239 days, and the case with the shortest survival was PDL1+immunoexcluded.

Conclusion
Most UCs are immune-inflamed and PDL1 expression is limited and not related to TIME cell types. A deeper characterization of T lymphocytes is necessary to further stratify the immune response.

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IMPLEMENTING AI IN DIGITAL HISTOPATHOLOGY: TOWARDS QUANTITATIVE ANALYSIS AND MACHINE LEARNING INTEGRATION IN DIAGNOSTICS AND RESEARCH

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Background
Histopathological analysis of tissue specimens is a cornerstone in diagnostics and research. The traditional approach for histopathological analysis involves manual examination by a pathologist, which is time-consuming, with certain degree of subjectivity, intra- and inter-observer variability. To counteract this, transition to digital pathology integrating artificial intelligence (AI) has been made. Therefore, the aim of this presentation is to give an overview of how machine learning and deep learning can be integrated in research projects and diagnostic applications and to compare this with manual analysis.

Materials & Methods
Machine learning and deep learning were used in different research and diagnostic contexts and compared to manual analysis. Some examples include object detection and classification of tissues (detection of tumor, necrosis, inflammation, fibrosis), cell count and positive cell detection in immunohistochemistry, classification of cell types in an inflammatory process, etc. which are critical tasks in histopathological image analysis. More complex analyses, such as tumor margin detection, and superposition of fluorescent and HE images were also performed.

Results
Benefits of integrating AI in research projects and diagnostic purposes include time savings, reduction of variability, and the ability to extract relevant information from large-scale histopathological datasets. Moreover, the overlaying of images enables the concurrent visualization of multiple markers, thereby enhancing comprehension of complex biological processes.

Conclusion
The utilization of AI in diagnostics and research unlocks boundless potential, as its capabilities are refined through continuous training. Its use enhances accuracy, efficiency, depth of analysis and reproducibility, while minimizing the risk of error.

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USING NEXT-GENERATION SEQUENCING AND IN SITU HYBRIDIZATION TO IDENTIFY THANATOTRANSCRIPTOMIC EXPRESSIONS OF MOUSE BRAINS FOR ESTIMATING POSTMORTEM INTERVALS

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Background
Forensic pathologists often need to estimate postmortem interval (PMI) for criminal investigations. Recently, attention has turned to thanatotranscriptomes, which reflect gene expression in response to micro-environmental changes after somatic death. The study aims to establish a connection between thanatotranscriptomes and PMI using next-generation sequencing (NGS) and in situ hybridization (ISH), proposing a novel method for forensic applications.

Materials & Methods
Seventy six-week-old female C57BL/6 mice were acclimatized for two weeks post-shipping, then euthanized. Carcasses were stored in still-air chambers at 25°C, and brain tissues were collected at ten time-points (up to 48 hours), with seven mice allocated per time-point for RNA sequencing and formalin fixation. NGS identified thanatotranscriptomes, and ISH targeted Casp3 and Bcl-6, quantifying expression levels. Reproducibility was tested eighteen months later.

Results
The NGS results showed that thousands of genes were dramatically upregulated over time in dying mouse brain tissues and could still be observed up to 48 hours postmortem. Among these expressed genes, several important groups were identified, including stress-, apoptosis-, and development-related genes. The expression trends of Casp3 and Bcl-6 were similar in both NGS and ISH, with correlation coefficients of 0.83 and 0.67, respectively. Bcl-6 ISH demonstrated topographic characteristics, with high expression in specific brain regions like the hippocampal pyramidal layer of CA1 and CA2. Up to 6,000 genes showed postmortem expression trends across two sampling sessions, with 4,000 exhibiting highly correlated patterns.

Conclusion
This study suggests thanatotranscriptomes as a novel PMI estimation method, indicating reproducible postmortem gene expression of stable genes and highlighting ISH's topographic transcriptomic insights.

ORAL ABSTRACTS

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INTESTINAL AND PERITONEAL LESIONS IN WOUNDED UMBILICAL OUTPOUCHINGS IN PIGS AFTER TRANSPORTATION

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Background
Umbilical outpouchings (UOs) in pigs are of welfare concern. One of the challenges is the transportation of these animals due to the size of the UOs and ulcerations on them. With certain precautions, pigs with UOs can be transported; however, UOs may be associated with intra-abdominal lesions, which may worsen during transportation. The prevalence and characterization of intra-abdominal lesions associated with UOs following transportation has not been investigated. The objective of the present study was to evaluate intra-abdominal lesions in slaughter pigs with wounded UOs following transportation to an abattoir.

Materials & Methods
A cross-sectional study involving three conventional Danish pig herds was conducted comprising 96 slaughter pigs with wounded UOs transported to an abattoir. The pigs were deemed eligible for transportation by a veterinary clinician. Following slaughter, the UOs with accompanying intestines were transported to the University of Copenhagen and examined pathologically.

Results
Three distinct morphological categories were present: hernia, enterocystoma and herniating enterocystoma. Intra-abdominal lesions were present in 72% of the animals, representing 65% of the hernias, 77% of the enterocystomas, and 100% of the herniating enterocystomas. Several different lesions were found, e.g. haemorrhages, peritonitis and hypertrophy of the intestinal muscular layers.

Conclusion
The prevalence of intra-abdominal lesions in pigs transported with a wounded UO was found to be high independent of the underlying condition, and it is clear that these pigs constitute a vulnerable group of animals, especially during physical stressful situations like transportation. Therefore, information is also needed regarding the prevalence of intra-abdominal lesions in UO-pigs without wounds on the UO.

POSTER ABSTRACTS

Toxicological Pathology

3 | ARTIFICIAL INTELLIGENCE-BASED HISTOPATHOLOGICAL ANALYSIS IDENTIFIES UNIQUE CORRELATES OF TUBERCULOSIS

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Introduction
Mycobacterium tuberculosis, the tuberculosis (TB) causing bacillus, infects 2 billion globally, with 8-9 million new cases and 1-1.5 million deaths annually. Aerosol infection leads to lung granulomas, complex structures which coordinate host defense, affect outcomes, and are commonly evaluated by visual examination using light microscopy. Here, we present a novel artificial intelligence (AI) model to assist pathologist examination by quantifying granuloma features and acid-fast bacilli (AFB). We (i) describe the AI model; (ii) demonstrate its application to hundreds of lung tissue sections allowing unquantifiable histopathology features to be automatically quantified; and (iii) conduct statistical analyses to identify granuloma-level correlates of disease.

Methods
A supervised AI model was created with Aiforia® Cloud 5.5, trained on 124 digital images from 8 independent experiments, representing at least 5 batches of stained tissue sections from M. tuberculosis-infected Diversity Outbred mice. After verification, the model was used to analyze ›800 mouse lung tissue sections, producing data for correlation analysis.

Results
The AI model (i) segmented tissue components and (ii) detected cell types, quantifying areas and numbers and (iii) spatial coordinates of objects. Data facilitated identifying granuloma-level correlates with host outcomes including host bacterial control, weight loss, and survival.

Conclusion
The AI-assisted, quantitative histopathology analysis provides a comprehensive, quantitative, and objective assessment of TB granulomas. This approach will help advance our understanding of TB pathogenesis; evaluate efficacy of novel vaccines and therapies; and in the future may aid in diagnosis of TB patients.

POSTER ABSTRACTS

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8 | A MECHANISTIC ONE-GENERATION RAT STUDY TO HIGHLIGHT THYROID DISRUPTION IMPACT ON DEVELOPMENTAL NEUROTOXICITY USING PATHOLOGY AND SPATIAL TRANSCRIPTOMICS ANALYSIS

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Introduction
Exposure to certain chemicals can interfere with thyroid hormone synthesis, secretion, or metabolism. As thyroid hormones (TH) are involved in brain development, altered TH levels in fetuses and newborns is a major concern.
Methods
Two TH disruptors were analyzed: a direct thyroid toxicant (propyl-thiouracil, PTU), and an indirect thyroid toxicant (5-pregnen-3β-ol-20-one-16α-carbonitrile, PCN). A one-generation study was performed with 18 pregnant rats per group treated with PTU (2.4 mg/kg/day) or PCN (300 mg/kg/day) from gestational day (GD) 6 to post-natal day (PND) 21. The following parameters were evaluated: TH levels in plasma, targeted gene expression in liver, thyroid, and brain, and hepatic enzyme activity (UGT). Also, thyroid, liver, brain, and cochlea histopathology analyses were performed at different timepoints, as well as immunohistochemistry labeling to describe neurons, microglia, glial cells, and oligodendrocytes distribution, brain spatial transcriptomics, and auditory reflex measurements.

Results
This study showed PTU treatment induced significant decrease in TH levels in dams and pups plasma. In pups, follicular cell hypertrophy in the thyroid associated with gene deregulation was observed as well as specific brain lesions (periventricular heterotopia, persistence of external granular layer in cerebellum), and disruption of auditory reflex linked with cochlea maturation deficit. PCN treatment induced no TH variation but a TSH increase in dams only, it also induced in pups hepatic enzyme increase in gene expression and activity, and no toxic effect in the brain.

Conclusion
The data generated will help to further understand the differences between direct and indirect thyroid toxicants on thyroid and liver function and the link to neurodevelopmental toxicity.

POSTER ABSTRACTS

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10 | RABBITS IN THE HEADLIGHTSCONFUSING ANATOMIC ACCOUNTS

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Introduction
In researching references for forthcoming book chapters, we came across inaccuracies in the literature describing rabbit anatomy. This may have consequences for interpretation of histological changes in rabbit tissues. Stomach and skin are standard tissues evaluated in regulatory toxicology studies. Previous authors described a non-glandular portion in the stomach and poorly documented distribution of sebaceous glands in the different locations of skin. We investigated both tissues in healthy rabbits.

Methods
Several areas in the cardia, pyloric antrum, and the area previously described as non-glandular stomach were sampled from male and female control rabbits. Skin samples from healthy adult control females were collected from different locations (dorsal/lateral head, pinna, base of the ear, dewlap, inguinal, axillary, lateral/dorsal thorax, abdomen).

Results
All areas of stomach evaluated were found to be glandular only. In most of the skin locations, sebaceous glands were scanty, individually distributed or in groups of up to 2 units. The only region with abundance of sebaceous glands diffusely distributed in groups of up to 8-10 units was the external pinna.

Conclusion
The rabbit stomach is glandular only. We think previous authors sampled the cardiac sphincter, as this is extensive and so well-developed that the rabbit cannot vomit. Distribution and density of skin sebaceous glands varies depending on location, which can have important implications in diagnosing spontaneous conditions such as sebaceous atrophy or adenitis, and test-item related effects.

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11 | ASSESSING BENZENE TOXICITY IN BONE MARROW WITH CYTOLOGY AND MACHINE LEARNING

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Introduction
Evaluating bone marrow toxicity necessitates both histopathological and cytological assessments. This study aimed to evaluate benzene-induced toxicity in bone marrow using conventional cytological techniques and to compare the classification of cellular lineages via machine learning-based image analysis of whole slide imaging (WSI) of femur samples.

Methods
Male C57BL/6 mice (n=40) were divided into four groups: group 1 served as the control, while groups 2, 3, or 4 received 6, 60, or 600 mg/kg of benzene, respectively. Benzene was orally administered twice daily at 7-hour intervals for 2 days, and animals were sacrificed 17 hours after the final administration. Blood analyses were conducted, and bone marrow parameters, including myeloid to erythroid (ME) ratio, were assessed cytologically and histopathologically using the left and right femurs, respectively.

Results
Several blood parameters exhibited significant alterations, including decreased white blood cells (WBC) and reticulocyte levels by benzene treatment. However, cytometric analysis of the femur indicated that benzene treatment increased WBC count in a dose-dependent manner. Machine learning-based image analysis of femur WSI successfully classified myeloid and erythroid cell lineages in the bone marrow, producing results closely matching manual counting by human observers, despite no alterations in cell density among the groups. Additionally, benzene exposure increased the ME ratio as analyzed by cytometric method, consistent with machine learning-based image analysis.

Conclusion
Overall, exposure to benzene led to alterations in various hematological and bone marrow parameters in mice. Integration of conventional cytometric assessment of femurs with machine learning-based image analysis facilitates precise categorization of cellular lineages, presenting potential for retrospective investigations utilizing WSI.

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13 | EVALUATION OF GENERAL TOXICITY AND SAFETY PHARMACOLOGY FOR A SARS-COV-2 SUBUNIT VACCINE IN RAT, RABBIT AND DOG

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Introduction
The SARS-CoV-2 pandemic has led to innovative approaches in vaccine development, including the creation of new viral vectors and mRNA-based vaccines. Despite these advances, uncertainties surrounding vaccines continue to cause concerns and fears about vaccine safety. Therefore, comprehensive preclinical toxicity evaluations are essential to establish the safety profiles of these vaccine candidates. This study investigates the toxicity profile of HuVac-19, a subunit vaccine for SARS-CoV-2 that targets the receptor-binding domain of the virus.

Methods
We conducted single and repeated dose toxicity and safety pharmacology studies in rats, rabbits, and dogs.

Results
In rats and rabbits, repeat-dose studies revealed reversible alterations in hematologic and serum biochemical markers within the adjuvant and/or vaccine-administrated groups. These changes were transient and returned to normal during the recovery phase. Additionally, reversible changes in the absolute and relative weights of specific organs like the prostate in rats and the thymus in rabbits were noted. Upon gross examination, the injection sites in rats and rabbits displayed discoloration and localized lesions, while histopathological analysis identified granulomatous inflammation, infiltration by inflammatory cells, and myofiber degeneration/necrosis. These inflammatory responses were localized, not associated with broader toxicological effects, and were resolved over time. In pharmacological safety assessments, which evaluated the neurobehavioral, respiratory, and cardiovascular systems, HuVac-19 did not induce any significant toxicological or physiological alterations.

Conclusion
Our findings suggest that HuVac-19 exhibits a favorable safety profile and supports its advancement to human clinical trials.

POSTER ABSTRACTS

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15 | ASSESSMENT OF REPRODUCTIVE AND DEVELOPMENTAL TOXICITY INDUCED BY PERFLUOROPENTANOIC ACID (PFPEA) IN SPRAGUE-DAWLEY RATS

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Introduction
Perfluoroalkyl and polyfluoroalkyl substances (PFAS), among them Perfluoropentanoic acid (PFPeA), are extensively utilized owing to their distinctive chemical attributes. PFPeA has been detected in various environmental matrices, including biota and human breast milk, prompting concerns regarding potential health implications.

Methods
This study aimed to assess the potential toxicity and to screen reproductive and developmental toxicity of PFPeA in Sprague-Dawley rats following repeated oral gavage administration, adhering to OECD testing guideline 422. Male and female rats were orally administered PFPeA at doses of 0, 30, 120, and 300 mg/kg/day for approximately 50 days (premating, mating, gestation, and lactation periods).

Results
In general toxicity assessments, males exposed to 300 mg/kg/day exhibited decreased red blood cell indices and increased reticulocytes, along with decreased thyroid hormone levels at 120 mg/kg/day or higher. The changes in red cell indices and reticulocytes observed in males were reversible, and no effects were observed on dosed females. Squamous cell hyperplasia in the stomach was observed at 300 mg/kg/day but was reversible. In the reproductive/developmental toxicity screening, F1 pups at 300 mg/kg/day showed skin paleness, decreased body weight, and reduced blood T4 concentration, indicating potential developmental toxicity.

Conclusion
In summary, while no significant general toxic effects were observed, both male and female neonates exposed to 300 mg/kg of PFPeA exhibited reproductive and developmental toxicological signs, including skin paleness, decreased body weight, and reduced blood T4 concentration.

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18 | ASSESSMENT OF COMPOUND-INDUCED HEPATOCELLULAR HYPERTROPHY IN RATS THROUGH DEEP LEARNING-BASED QUANTITATIVE ANALYSIS

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Introduction
In preclinical studies, hepatocellular hypertrophy is frequently encountered in rodent liver histology. The possible challenges with traditional approaches to interpreting hypertrophy may lack uniformity, and could be influenced by scale shifts and subjectivity. Variation in defining treatment-related hypertrophy exists across studies due to factors like strains, age, and sex. The study developed an AI model quantifying cell hypertrophy in rats in HE-stained whole slide images.

Methods
A supervised AI model was trained to segment liver structures and calculate the area, number and circumference of hepatocytes in different zonal regions of the liver. The model was trained employing semantic and instance segmentation, along with object detection convolutional neural networks on 98 HE-stained rat tissue images. The model conducted an analysis on liver sections from rats exposed to Carbamazepine, enabling correlation assessments and comparisons with pathologist evaluations across various treatment cohorts.

Results
The AI model demonstrated high accuracy in discerning cell hypertrophy variances in the dose groups. It established centrilobular hypertrophy with a significant correlation between centrilobular mean and median areas, alongside hepatocyte circumferences, with pathologist observations and grading, and facilitated the establishment of cutoffs. Spatial analysis provided the distribution of hypertrophic cells within the tissue microenvironment, quantifying the degree of hypertrophy in periportal, midzonal and centrilobular locations.

Conclusion
The AI model provides a reliable and rapid solution to quantify hypertrophic liver changes, aiding in understanding compound-induced microscopic findings. It accurately interprets hepatocyte hypertrophy, including zonal distribution. Incorporating AI into pathological workflows is expected to offer extra decision-making assistance for pathologists and enhance consistency.

POSTER ABSTRACTS

Toxicological Pathology

19 | CASE REPORT: SPONTANEOUS COAGULATING GLAND ADENOCARCINOMA IN A SPRAGUE-DAWLEY RAT

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Introduction
Although it is known that some chemicals can induce prostatic tumors in rats, spontaneous occurrence is rare, especially, coagulating gland adenocarcinomas have not been reported in the literature. In this report, we present a case of spontaneous coagulating gland adenocarcinoma in a Sprague-Dawley rat.

Methods
This case was a male 110-week-old Crl:CD(SD) rat allocated to the mid-dose group of a 104-week carcinogenicity study. No abnormal findings were observed in the clinical observation and at routine necropsy, but there were microscopic proliferative findings in the coagulating gland. Then, serial sections were stained with hematoxylin and eosin, histochemical staining and immunohistochemical staining (IHC).

Results
Histologically, a neoplastic finding was in the coagulating gland, involving the entire coagulating gland. The tumor cells were arranged in irregular duct-like or small alveolar patterns. The cells had pale round nuclei with distinct nucleoli and abundant eosinophilic cytoplasm with marked pleomorphism and anisokaryosis. There were few mitotic figures. Abundant fibrous connective tissue and fibroblast-like spindle cells were found around the tumor cells in the stroma. Watanabe's method for reticulum and Masson's trichrome staining showed abundant collagen fibers in the stroma. Neutrophil infiltration was observed in the glandular lumen and stroma. IHC showed that the tumor cells were positive for pan-cytokeratin and negative for vimentin. Spindle stromal cells were positive for vimentin and alpha smooth muscle actin. No related changes were found in the other organs including accessory glands or urinary tract.

Conclusion
Based on the results of the histopathological examinations, we diagnosed the finding as a spontaneous coagulating gland adenocarcinoma.

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21 | AUTOMATED ASSESSMENT OF NECROTIC NEURONS AND DARK ARTIFACTUAL NEURONS IN TRANSGENIC MOUSE MODEL OF ALZHEIMER'S DISEASE USING DEEP LEARNING

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Introduction
Neuronal necrosis is an end-stage, non-reversible cellular response to injury. Neuronal necrosis is considered as an adverse finding and therefore identifying the presence of necrotic neurons is an important endpoint in routine preclinical toxicology studies, animal model phenotyping, or in specialized neurotoxicity studies. The main differential for neuronal necrosis in HE-stained brain sections are dark neuron artifacts, which are common histological artifacts and potentially difficult to differentiate from true neuronal necrosis.

Methods
A supervised deep learning object detection AI model was trained to detect necrotic neurons and dark neuron artifacts in HE-stained mouse brain sections. An additional AI model was trained to detect necrotic neurons in Fluoro-Jade C (FJ)-stained brain sections. Brain sections from 20 transgenic mice and 3 age-matched wild-type controls were analyzed with the AI model, and the results of HE and FJ-stained images were compared.

Results
The AI model accurately identified the number and spatial locations of hippocampal necrotic neurons and differentiated them clearly from artifactual dark neurons in HE and from auto-fluorescent cells in FJ with low error. A heatmap incorporated in the AI model supported the pathologist in detecting the areas with the highest necrotic neuron and dark neuron densities.

Conclusion
AI-based detection of necrotic neurons in HE-staining aligned with FJ-staining results, providing a supportive tool for pathologists to locate and count the necrotic neurons in HE-stained images, and differentiate those from artifactual dark neurons. The current AI model can improve efficiency and increase objectivity in neuropathology studies through time-saving and decreased inter-pathologist variation.

POSTER ABSTRACTS

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22 | ARTIFICIAL INTELLIGENCE BASED NUCLEAR DENSITY MAPS FACILITATE HEPATOCELLULAR HYPERTROPHY DETECTION ON A WHOLE SLIDE VIEW

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Introduction
Computer-aided diagnostics (CAD) can support the workflow of toxicologic pathologists. Xenobiotic-induced hepatocellular hypertrophy is a common microscopic finding observed in toxicology studies and its consistent and efficient evaluation is challenging. Hepatocyte size can be estimated by AI nuclear segmentation and whole-slide level density maps. This study aimed to develop and validate an AI-based CAD method using automated detection of hepatocellular nuclei and nuclear density maps to assist the toxicologic pathologists in the detection of xenobiotic-induced hepatocellular hypertrophy.

Methods
Hematoxylin and eosin (H&E) stained rat liver sections were digitized at 40x magnification. The Patholytix AI Workflow (Deciphex Ltd, Dublin Ireland) was used to create annotations and to train a convolutional neural network (CNN) to detect hepatocellular nuclei. Studies included Sprague-Dawley and Wistar rats with durations ranging from 3-90 days. Detection of hepatocellular hypertrophy on AI-derived nuclear density maps were compared to toxicologic pathologist's diagnosis on studies not used for training the classifier. Apart from hypertrophy, the studies included slides with hepatocyte glycogen vacuolation, lipid vacuolation and sinusoidal congestion.

Results
Nuclear density maps facilitated detection of hepatocellular hypertrophy. Other liver alterations with lowered nuclear density were also identified with the tool.

Conclusion
AI-derived nuclear density maps can support detection of xenobiotic-induced hepatocellular hypertrophy in rats at the whole slide level but pathologist assessment at higher magnification remains essential to confirm the diagnosis and differentiate hypertrophy from other lesions affecting nuclear density. Further refinements of the model that would differentiate alterations with lower nuclear density that are unrelated to hypertrophy are being evaluated.

POSTER ABSTRACTS

Toxicological Pathology

23 | TRAINING AND EVALUATING MASK R-CNN ALGORITHM FOR DETECTION OF COMMON HISTOPATHOLOGY FINDINGS IN RAT KIDNEY

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Introduction
In light of recent advancements in artificial intelligence (AI) technology, investigations have been undertaken to explore the capacity of AI in discerning and categorizing pathological findings. This study aims to train and evaluate the performance of an artificial intelligence model to detect frequently observed spontaneous findings from kidneys in rat subacute toxicity studies, such as mononuclear cell infiltration (MCI), basophilic tubules (BT), proteinaceous cast (PC), tubular dilation (TD), mineralization (MZ), and hyaline droplet (HD).

Methods
Up to 2670 annotations for each microscopic finding were utilized, employing the Mask R-CNN algorithm. Model training was conducted using two Geforce RTX 3090 GPUs under conditions including 9X augmentation, 0.005 learning rate, 200 epochs, SGD optimizer, and 16 batch size. Accuracy was derived from the ratio of the number of images that the model successfully detected among the test set images.

Results
Following training, the model achieved an accuracy of 88.68%. Subsequently, attempts were made to detect findings in fifty kidney slide images using the model's weights. The Spearman correlation coefficient was calculated by comparing the model-detected finding areas with the severity score, gold standard, evaluated by an experienced pathologist, resulting in coefficients of 0.886 for MCI, 0.899 for BT, 0.860 for PC, 0.761 for TD, 0.712 for MZ, and 0.719 for HD, indicating significant similarity ($P<0.05$).

Conclusion
In conclusion, as the high correlation between the finding areas detected by the model and severity score evaluated by pathologist were seen, this AI model shows promise in assisting pathologists in evaluating kidney tissue in rat subacute toxicity studies.

POSTER ABSTRACTS

Toxicological Pathology

24 | CARDIAC SINGLE CELL DEGENERATION IN COMMON MARMOSETS (CALLITHRIX JACCHUS)

S.R. Hartung[1], **A.K. Haverkamp**[1], **A. Romeike**[1]
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Introduction
In the last two decades, common marmosets (Callithrix jacchus) have been more frequently used in preclinical toxicity studies due to their physiological similarities with humans. For instance, several surface receptors on marmoset lymphocytes are cross-reactive with monoclonal antibodies against the corresponding human variant and the hepatic metabolism mimics the human pathways more closely than other non-human primates (NHPs). Nevertheless, marmosets differ from macaques regarding spontaneous diseases and background pathology what might complicate the interpretation of findings in this species.

Methods
52 common marmosets used as control animals in preclinical toxicity studies underwent a full macroscopic examination with organ weight collection at necropsy. Routinely processed HE stained standard sections of the hearts were examined by light microscopy.

Results
Histological findings included mononuclear cell infiltrates (51.9 %; 0-100 %), myocardial fibrosis (21.2 %; 0-75 %), and minimal to slight single cell degeneration/necrosis (19.2 %; 0-37.5 %) of focal to multifocal distribution with varying incidences per study. The degeneration/necrosis was characterized by hypereosinophilia, loss of cross striation, vacuolation, and/or fragmentation of individual cardiomyocytes and was only rarely accompanied by minimal, neutrophilic infiltration.

Conclusion
Histological findings in the heart of common marmosets are prevalent in preclinical toxicity studies. While mononuclear cell infiltrates and myocardial fibrosis occur at similar incidences in macaques, single cell degeneration/necrosis is infrequently observed in other NHPs. The finding has been previously mentioned by other authors and a relationship to stress was suggested but not proven. Herewith, the authors strive to put attention to an observation in common marmosets which can be easily misinterpreted as cardiac toxicity.

POSTER ABSTRACTS

Toxicological Pathology

25 | MYSTERIOUS LESION IN THE BRAIN OF A CYNOMOLGUS MONKEY (MACACA FASCICULARIS): WHAT'S YOUR DIAGNOSIS?

A.K. Haverkamp[1], S.R. Hartung[1], A. Romeike[1]
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Introduction
In recent years, the development of new drug modalities has underlined the importance of nonhuman primates in biomedical research and the central nervous system has gained importance as a target for different modalities. Herewith, the authors describe an unusual finding in the brain of a monkey of unknown cause.

Methods
Cynomolgus monkeys from a single dose toxicity study underwent terminal procedures. Tissue specimens were processed to block, sectioned, and stained with H&E. Special staining and immunohistochemistry were applied on selected brain specimens.

Results
The occipital lobe of one animal revealed a locally extensive loss of structure with formation of irregularly sized, eosinophilic, granulated globules; an irregular, peripheral rim of large, polygonal cells (LPCs) with granulated cytoplasm and vesicular nuclei; and prominent deposition of perivascular pigment (Perl`s positive). The LPCs stained positive with Pan-Cytokeratin and the granular material within the cytoplasm of these cells reacted with PAS and Grocott. A milder, inconsistent, positive staining with PAS and Grocott was also noted within the globular structures. Immunohistochemistry for GFAP, S-100, and Iba-1 was conducted but labeled neither the peripheral LPCs nor the globular structures.

Conclusion
A relationship to the test article was considered unlikely based on morphology, chronicity, and occurrence in a single animal but the cause and histogenesis of the finding remain uncertain. The expression of Pan-Cytokeratin confirmed an epithelial origin for parts of the lesion and a congenital malformation was considered but is not fully compliant with all of the observed features. What's your diagnosis and/or what are your suggestions for further examinations?

POSTER ABSTRACTS

Toxicological Pathology

26 | EXPANDING THE CHARACTERIZATION OF BASAL NUCLEI LESIONS IN BEAGLE DOGS: AN IMMUNOHISTOCHEMICAL APPROACH

S.D. Cahalan[1], L.A. Obert[2], J.M. Kreeger[2], C.E. Fishman[2], F. Noor[1]
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Introduction
Following the identification of bilateral basal nuclei lesions in Beagle dogs by Hempel et al, 2024, our investigations will expand upon their lesion description using a broader panel of immunohistochemical markers that will further characterize the initial findings of astrocytosis, microgliosis, and myelin edema and degeneration.

Methods
Affected and control Beagle dog brains will be analyzed via an expanded set of immunohistochemical markers of neuronal and glial identity/integrity, synaptic density, and cell death/injury mechanisms which include NeuN, Synaptophysin, TUNEL, Fractin, GFAP, Iba1, Olig2, Myelin Basic Protein, and various neurotransmitters.

Results
Changes in neuronal and glial density and synaptic integrity are anticipated, which will highlight the extent of cellular degeneration within the lesions. Cell death markers, TUNEL and Fractin, might also elucidate the predominant pathways of cell death/injury in the affected brain regions.

Conclusion
This study is poised to build upon the foundational work of Hempel et al., offering novel insights into the complex nature of the basal nuclei lesions in Beagle dogs. By expanding the scope of immunohistochemical characterization, we aim to uncover the pathogenesis of these lesions, thereby enhancing the differentiation between spontaneous and test article-induced changes in preclinical safety assessments.

POSTER ABSTRACTS

Toxicological Pathology

28 | GLYCOPROTEIN NON-METASTATIC MELANOMA B (GPNMB) - A BIOMARKER FOR NEUROINFLAMMATION IN PRECLINICAL MODELS

I.M. Langohr[1], D. Siddappa Thimmanahalli[1], P. Bathala[1], M. Kramer[1], Y. Chen[1], M. Levit[1], L. Smith[1], K. George[1], A. Flier[1], J. Nietiupski[1], C. Shapiro[1], T. Taksir[1], R. Riley[1], C. Morel[1], B. Zhang[1], D.S. Bangari[1]
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Introduction
Lysosomal storage diseases (LSDs) are inherited disorders of lysosomal function that result in progressive accumulation of metabolic byproducts and subsequent tissue pathology. Mouse models of human LSDs recapitulate the disease pathophysiology and serve as critical drug discovery tools. To further explore the translational utility of these preclinical models, we studied the expression of glycoprotein non-metastatic melanoma B (GPNMB) protein as a disease biomarker in Niemann-Pick type C (NPC), Sandhoff disease, metachromatic leukodystrophy (MLD), and acid sphingomyelinase deficiency (ASMD) mouse models. GPNMB has been linked to lysosomal integrity and has also been implicated in neuroinflammation due to its high expression in macrophages and microglia.

Methods
Plasma, cerebrospinal fluid and brain samples from diseased mice and age-matched wild-type mice were evaluated by quantitative real-time PCR, ELISA, proteomics, and immunohistochemistry for murine GPNMB.

Results
We observed increased GPNMB mRNA and protein levels in the plasma, cerebrospinal fluid, and brain of all four mouse models. GPNMB immunoexpression localized within substrate-laden foamy macrophages in the brain of these mice whereas wild-type mouse brains were devoid of GPNMB-immunopositive cells. In NPC mice, increases in plasma and tissue GPNMB expression were age-dependent, indicating its role as a marker of disease progression.

Conclusion
Our results validated the involvement of GPNMB-immunopositive macrophages in LSD pathophysiology. Therefore, GPNMB may be a useful biomarker for tracking progression of LSD-related neuroinflammation and assessing the efficacy of experimental therapeutics.

POSTER ABSTRACTS

Toxicological Pathology

29 | MELANOSIS OF THE DENTATE NUCLEUS IN TWO CYNOMOLGUS MACAQUES

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Introduction
Melanin deposits occasionally occur in central nervous system tissues. In cynomolgus macaques, spontaneous perivascular melanin pigment occurs in the meninges and in the cerebral cortex. Additionally, neuromelanin pigment accumulates with age in the substantia nigra and locus coeruleus in human and non-human primate brains. We report pigmentation consistent with melanin specifically within the cerebellar dentate nucleus of two juvenile cynomolgus macaques. Melanosis in the dentate nucleus has been reported in people but to our knowledge has not been reported in non-human primates.

Methods
Two 18-month-old male Chinese origin cynomolgus macaques were part of a study in which animals received a single dose of an adeno-associated virus gene therapy via direct brain administration, remote from the cerebellum. On study, animals had regular detailed clinical observations and had neurologic assessments. Euthanasia occurred at scheduled study termination, 58 days post treatment.

Results
There were no notable in-life findings. At necropsy, both animals had gross observations of symmetrically bilateral black discoloration in the cerebellar white matter. Microscopically, this correlated to dark brown granular pigment in the neuropil of the dentate nucleus of the cerebellum. The pigment was not within neuronal soma.

Conclusion
Pigmentation of the dentate nucleus in these two macaques closely resembles melanosis of the dentate nucleus reported in people. The underlying pathogenesis of this condition is unknown. No relationship to the test article was determined in this study. This may represent a rare spontaneous condition in cynomolgus macaques.

POSTER ABSTRACTS

Toxicological Pathology

30 | ENABLING RAT BONE MARROW CELL LINEAGE IDENTIFICATION IN H&E WHOLE SLIDE IMAGES USING MULTIPLE IHC-GUIDED DEEP LEARNING MODELS

E.A.R.P. Rios Piedra[2], I.B.V. Berenguer Veiga[1], M.T. Tecilla[1], K.H. Hahn[1], P.M. Maliver[1], J.E. Eastham[2], A.O.A. O. Adedeji[2], S.L .Lau[2], M..B Baca[2], R.S. Sullivan[2]
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Introduction
Hematoxylin and eosin (H&E)-stained bone marrow (BM) tissue sections are routinely assessed in toxicologic pathology studies. Although evaluation can be augmented with immunohistochemical (IHC) labeling, IHC is not a standard practice for routine BM assessment. We hypothesized that we could develop computational approaches to predict IHC labeling patterns of cells on H&E-stained BM tissue sections using deep learning (DL) algorithms trained on IHC-informed H&E-based ground truth to enhance routine evaluation of BM cell lineages.

Methods
We developed a DL-based segmentation pipeline to predict cellular IHC-expression patterns on H&E slides. Rat formalin-fixed paraffin-embedded decalcified sternum slides were stained and de-stained to generate H&E and IHC from the same section. Whole slide images (WSI) were acquired and aligned (registered) to enable pathologist-generated multimodal ground truth. Then, the IHC-expression model was trained on the H&E slides and IHC labels, developing an independent model for each IHC marker. Results were aggregated and segmentations created for each cell in the marrow section, providing an estimate of IHC expression and other endpoints (e.g., cell density). Endpoints can be made available to enhance results interpretation and communication.

Results
N.A.

Conclusion
We successfully developed an AI-enabled method to augment the utility of H&E WSI analysis with predicted IHC marker expression results. This methodology may extend routine BM analysis, trigger additional studies (e.g., BM smears cytology) to further investigate cell population shifts, increase pathologists' confidence when diagnosing BM pathology samples, and provide quantitative data for results visualization and communication.

POSTER ABSTRACTS

Toxicological Pathology

31 | COMPARATIVE EXOCRINE GLAND HISTOLOGY IN LABORATORY RODENTS

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Introduction
Glandular structures play a crucial role in the physiological processes of laboratory rodents but not all are routinely evaluated in toxicology studies. This study aims to elucidate the morphological histology of exocrine glandular tissues across the rodent body.

Methods
Formalin fixed paraffin embedded tissues stained with hematoxylin and eosin from four RccHan™:WIST rats and four CD 1 mice were used (male/female, 10-12 weeks and 104 weeks of age). These included the Harderian, Meibomian, extraorbital lacrimal glands, glands of the nictitating membrane, salivary, Ebner's, Zymbal's, clitoral, preputial, circumanal, sebaceous and apocrine glands from mice and rats.

Results
A comprehensive set of histology images and sections were compiled to facilitate a deeper understanding of the gland anatomy in laboratory rodents.
Upon examination, the different glands revealed distinct histological features. Sebaceous, mucous, serous or mixed glands appear as simple or compound glands. Compound glands contain multiple branched ducts. Furthermore, they are divided into tubular or alveolar glands, and may also be divided into tubuloacinar and tubuloalveolar glands. Characteristic features for different cell types are established, e.g., sebaceous glands display a characteristic acinar structure with lipid-filled cells, while the salivary glands display a tubuloacinar morphology with serous and mucous cells in varying proportions.

Conclusion
Although the histological classification of glands follows known principles, it might be difficult to distinguish the different gland types. This collection should contribute to a better understanding of interspecies histological variance, which is essential for translational research and toxicological assessments.

POSTER ABSTRACTS

Toxicological Pathology

33 | HISTORICAL CONTROL DATA FROM LABORATORY NEW ZEALAND WHITE AND DUTCH BELTED RABBITS (ORYCTOLAGUS CUNICULUS)

D. Corbetta[1], K. Isobe[1], R. Masood[1], P. Rogerson[1], A. Bradley[1]
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Introduction
A retrospective study was performed to determine the incidences of spontaneous findings in New Zealand White (NZW) and Dutch Belted (DB) rabbits.

Methods
A total of 2,170 NZW (526 males/1,644 females) and 100 DB control rabbits (50 animals per sex), aged between 4-7 months from 158 studies evaluated between 2013-2022 were assessed. Routes of administrations were oral gavage, intra-muscular, intra-venous, intra-arterial, subcutaneous, ocular(topical) instillation, intraocular(intra-vitreal;intra-cameral), intra-dermal, intra-vaginal, intra-articular, intra-nasal. There was a total of 28 ocular studies.

Results
Mixed inflammatory cell infiltration in the lung was the most recorded finding in NZW (19.0% females/12.0% males) and DB (38.0% females/22.0% males) rabbits. Other common lesions were renal tubular mineralization (NZW=16.3% females/14.6% males;DB=6.0% females/4.0% males), renal tubular basophilia (NZW=15.1% females/11.4% males;DB=16.0% females/10.0% males), hepatic mononuclear cell infiltrations (NZW=10.5% females/7.1% males;DB=2.0% females/0.0% males), renal mononuclear cell infiltrations (NZW=4.7% males; 2.8% females;DB=14.0% females/10.0% males). In ocular studies the most common changes were rosette formation (NZW=7.5% females/4.4% males;DB=0.0% females/0.0% males), needle tract lesions (NZW=2.4% females/2.3% males;DB=14.1% females/14.1% males), mononuclear i
nfiltration (NZW=3.2% males/3.0% females;DB=17.2% males/10.9% females), lens degeneration (NZW=0.2% males/0.0% females;DB=14.1% females/7.8% males).

Conclusion
To our knowledge this is the most recent comprehensive study of background lesions and should facilitate differentiation between spontaneous and induced lesions in safety studies. Rabbits represent an excellent replacement to dogs as nonclinical species for toxicology studies.

POSTER ABSTRACTS

Toxicological Pathology

34 | SPONTANEOUS MONONUCLEAR CELL INFILTRATES IN THE BRAIN AND SPINAL CORD OF RODENT AND NON-RODENT LABORATORY SPECIES

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Introduction
The interpretation of mononuclear cell infiltrates (MCI) in the central nervous system can be challenging in nonclinical studies. A retrospective study was performed to determine the incidences of spontaneous MCI in the brain and spinal cord of rodent and non-rodent species.

Methods
Historical control data (HCD) of MCI from brain and spinal cord were collected from Sprague-Dawley and Han Wistar rats, CD-1 mice, beagle dogs, Göttingen minipigs, cynomolgus monkeys, and New Zealand White rabbits from studies evaluated between 2014 and 2024.

Results
The cynomolgus monkey was the nonclinical species with the highest incidence of MCI in the brain. Perivascular MCI in the brain were most recorded in monkeys (7.84% females / 9.23% males) and minipigs (3.50% females / 3.43% males) followed by dogs (3.22% females / 3.21% males), rabbits (0.24% females / 0.00% males), mice (0.17% females / 0.02% males) and rats (0.04% females / 0.06% males). In rodents, Sprague Dawley rats showed higher incidence of infiltrates in the brain than Han Wistar rats, and were mostly seen in the choroid plexus, meninges, and pineal gland of control males. In all non-rodent species, choroid plexus and meninges were the most common localizations of infiltrates in the brain. Findings in the spinal cord showed low incidences in all the species.

Conclusion
MCI in the brain and spinal cord are substantially most common in monkeys, minipigs and dogs compared to rabbits and rodents. This HCD review will help in the interpretation of these findings in toxicological safety studies.

POSTER ABSTRACTS

Toxicological Pathology

35 | INTESTINAL TUMOR GRADING IN BIOMEDICAL RESEARCH - EXPECTATIONS VS. REALITY

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Introduction
Mouse models remain the gold standard for in vivo research on human colorectal cancer (CRC). In our comparative experimental pathology lab, affiliated with cooperative research centers investigating intestinal microbiota, immunology, and carcinogenesis, we process 600 projects a year, with emphasis on translational oncology research. Discrepancies between the researchers' expectations and pathological evaluation are noted, especially with regard to end-point development of invasive carcinomas in mice.

Methods
A retrospective analysis of submitted formalin-fixed paraffin-embedded (FFPE) material histologically processed for haematoxylin-eosin (HE) stainings was performed. In total, 215 mice of different models (Azoxymethane (AOM)/ Dextran Sodium Sulfate (DSS); endogenous models) for intestinal carcinogenesis in the large and small intestine were investigated. Sampling technique varied (swiss rolls, tumors, parts of the intestine(s)). Slides were digitized and assessed by both veterinary and human pathologists. Lesions were graded according to the current INHAND (International Harmonization of Nomenclature and Diagnostic Criteria for Lesions) classification.

Results
We graded 190 high-grade adenomas, 83 carcinomas, 68 atypical hyperplasias, and 44 low-grade adenomas on HE slides. Most mice developed multiple tumors; ~ 25 % had carcinomas. In 82 samples, no lesion was detected. Overall, 51 % of all lesions were located in the small intestine, 49 % in the colon. A recurrent problem was inappropriate sampling, not allowing for reliable evaluation.

Conclusion
Tumor histology and grading in mice differs compared to humans. It is important to note that human and mouse carcinomas vary regarding their invasiveness. Expertise and awareness of the pitfalls (e.g., background lesions) is necessary to distinguish between pre-neoplastic and carcinomatous lesions.

POSTER ABSTRACTS

Toxicological Pathology

37 | SINGLE-CELL RARE EVENT DETECTION FOR CELLULAR LESION ASSESSMENT AND QUANTIFICATION USING DEEP LEARNING APPROACHES

H.W. Williams[1], E.C. Crouch[2], J.K. Kuklyte[1], L.L.B. Lord Bissett[1], L.B. Bertand[2], D.R. Rudmann[2], P.M. Moulin[1], S.M. Marcu[1]
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Introduction
Accurate identification of rare findings in rat liver, for example, single-cell necrosis and mitotic-figures, is critical in the histologic evaluation of toxicologic pathology studies. However, visual grading rare findings can be time-consuming and may be aided by quantitative analysis. Deep learning based object detection (OD) models can be trained with rare findings annotations on whole slide images (WSIs) to efficiently detect rare findings on unseen slides in a standardised manner.

Methods
Pathologists annotated 1,200 examples of necrotic/apoptotic and mitotic cells across 225 liver WSIs and 19 studies. Two species were represented by this annotation set, Han Wistar and Sprague Dawley. Classifiers were trained and evaluated using predictive masks on annotated and unannotated studies both qualitatively by a pathologist, and quantitatively by the data science team. False positive or false negative detections were correctly annotated to improve classifier detection.

Results
The developed classifier is evaluated on three generalisation levels: 1) tile, 2) slide, and 3) study. Up to 15% of the annotated data was set aside as a blinded validation set; the sensitivity (recall), precision and F1 score of the classifier was calculated on this data, to dtermine classifier accuracy. Results on unseen study data show generalisation capabilities of the solution to scanner, tissue processing, or other variations. Density of the detections is also estimated on full WSI images to qualitatively assess the specificity of the results.

Conclusion
This methodology enables reliable and swift detection of individual mitotic and necrotic/apoptotic cells in rat liver slides, as well as precise computer-assisted quantification. The approach could also be expanded to other species and tissue types with minimal requirement for additional data.

POSTER ABSTRACTS

Toxicological Pathology

38 | DEVELOPMENT OF AN AI-BASED DECISION SUPPORT TOOL MODEL FOR THE DETECTION OF INFLAMMATORY CELL INFILTRATION IN RAT BRAIN

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Introduction
Histopathological assessment of the brain is part of the standard evaluation performed in non-clinical safety studies. The most common microscopic finding observed in rat short-term studies is inflammatory cell infiltration. A convolutional neural network (CNN)-based deep learning model was developed to assist in efficient and consistent microscopic identification of inflammatory cell infiltrates in the brain.

Methods
A deep learning algorithm was trained on whole slide images (WSI) of hematoxylin and eosin stained sections scanned at 40x magnification using the Patholytix Preclinical platform (Deciphex). Three classes were created: inflammatory cell infiltrates, haemorrhage/congestion, and normal brain tissue. A CNN classifier was developed to generate inference masks, which were assessed both quantitatively (confusion matrices and metrics) and visually.

Results
Performance on blinded tiles showed F1 score of 0.93 and 0.97 for inflammatory cell infiltration and haemorrhage/congestion classes, respectively. When the full slides were blinded, the performance dropped to 0.67(+/-0.25) and 0.92(+/- 0.07) showing high stability of heamorrhage/congestion class detection, and reduced performance of infiltration class which shows that there is a need for increased diversity in training data for this class. The classifier predicted the normal class with an accuracy of 99%.

Conclusion
The CNN classifier discriminates normal tissue and inflammatory cell infiltrates. Yet, while the classifier generalizes very well when detecting the haemorrhage/congestion, the generalization capabilities are to be further investigated and improved for inflammatory cell infiltrates. Continuous refinement and expansion of the tool will enhance the toxicologic pathologists' workflow and improve brain lesion detection and assessment in non-clinical studies.

POSTER ABSTRACTS

Toxicological Pathology

39 | PERIPHERAL NEUROPATHOLOGY IN CYNOMOLGUS MONKEYS AFTER INTRAVENOUS INJECTIONS OF AN ANTICANCER OLIGONUCLEOTIDE

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Introduction
An oligonucleotide was developed as an anticancer drug and 4-week toxicity studies were performed in cynomolgus monkeys to identify the highest non-severely toxic dose (HNSTD).

Methods
Sixty monkeys received the vehicle control or the oligonucleotide at 4 dose levels via intravenous route once weekly for 4 administrations followed by a 4-week recovery. Microscopic examinations, in situ hybridization and immunohistochemistry (complement factors C3/C3b, C5a, C5b9 and Bb, also measured in blood) were performed on formalin-fixed tissues (brain, spinal cord, sympathetic and dorsal root ganglia, peripheral nerves, liver, kidney and lymph nodes).

Results
Transient increased C5b9 and Bb levels were noted at all doses. Neuronal necrosis, chromatolysis and mononuclear cell infiltration were observed in the sensory and autonomic ganglia at all doses with secondary axonal degeneration in the peripheral nerves, spinal cord (dorsal funiculi) and brain (pons). Basophilic granules in macrophages (liver) were noted. After the recovery period, no reversibility in nervous organs was observed, except at the low dose (partial). The test article was detected by ISH techniques in the liver, kidney and lymph nodes but not in the nervous tissues. Increased C3/C3b and C5a immunostaining was observed in the ganglia, nerves, spinal cord and pons.

Conclusion
In absence of clinical signs, functional changes and neuronal necrosis in the recovery animals, the low dose was considered the HNSTD. Microscopic lesions in nervous ganglia were described here for the first time with systemic administration of an oligonucleotide, most likely as a result of complement activation.

POSTER ABSTRACTS

Toxicological Pathology

41 | AI-ASSISTED QUALITY CONTROL FOR ARTIFACT DETECTION: DEPLOYMENT IN A GLP-COMPLIANT IMAGE MANAGEMENT SYSTEM

D. Rodrigues[1], C. Babcock[1], I. Nearchou[1], K. Latimer[1], C. Habermehl[1], A. Guaba[1], V. Ovtcharov[1], P. Caie[1]
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Introduction
Artifacts can arise throughout slide preparation and scanning. The presence of artifacts can impact the qualitative review of whole-slide images (WSI) and lead to false positive and negative results in image analysis pipelines. Quality control (QC) of digital slides is imperative, however manual assessment is laborious and time-consuming. Tools to automate this procedure and increase its reproducibility are urgently required. Here we present a Good Laboratory Practices (GLP)-compliant digital peer-review workflow for digital image QC using HALO Link as an Image Management System and SlideQC for automated AI-assisted artifact detection and segmentation.

Methods
SlideQC was developed to detect artifacts including air bubbles, dust/debris, folds, out-of-focus, and pen marker in Haematoxylin and Eosin and Immunohistochemistry stained WSI. SlideQC can be run in batch from HALO Link across entire slide sets for a GLP-compliant QC workflow. Workflow improvements in time savings compared to manual QC were investigated, including the time to delineate exclusion artifact regions for subsequent analysis.

Results
SlideQC analysis can be triggered from HALO Link for an AI-assisted GLP-compliant QC workflow. With HALO Link's GLP-compliance add-on, system-wide auditing is available for all actions that trigger data generation, modification, or deletion. Using SlideQC for automated QC resulted in time savings ranging from 29.6 to 50.0% compared to manual QC.

Conclusion
SlideQC provides a scaffold that guides the review and identification of artifact regions, assisting the QC process. SlideQC can add consistency and efficiency to the QC workflow and can be used in combination with HALO Link's auditing capabilities for a GLP-compliant workflow.

POSTER ABSTRACTS

Toxicological Pathology

42 | VANADIUM EXPOSURE EFFECTS IN THE RAT OLFACTORY BULBS

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Introduction
Epidemiological evidence suggests that prolonged exposure to environmental neurotoxic metals, including vanadium, may be linked to the development of neurodegenerative diseases and olfactory impairment. The exact mechanisms by which neurotoxic metals affect the olfactory system and contribute to neurodegenerative diseases are not fully understood. However, it is assumed that these metals may induce oxidative stress, inflammation, and neuronal damage in the olfactory bulbs. The aim of this study was to determine the effects of vanadium in the rat's olfactory bulbs.

Methods
Fourteen adult Wistar rats were divided in two groups. One group (n=7) was exposed to a solution with a concentration of 273 µg of vanadium pentoxide (V_2O_5) by intranasal delivery, the other group served as control (n=7). All animals received in each nostril 30 µL of solution, three times a week for 4 weeks. After exposure, rats were sacrificed. Full necropsy was performed, and the brains processed for routine paraffin embedding and immunohistochemistry to evaluate tyrosine hydroxylase (TH) and glial fibrillary acidic protein (GFAP) expression.

Results
Vanadium exposure resulted in gliosis and neuronal death in the olfactory bulbs. The glomerular layer displayed reduced TH expression whereas GFAF expression was higher in the same region of exposed rats.

Conclusion
Vanadium has a negative impact in the olfactory bulbs, compromising TH expression, which suggests impaired dopaminergic function, while inducing proliferation and activation of glial cells, as shown by GFAP expression. The latter process may involve a reactive neuroinflamation response to the injury caused by vanadium exposure.

POSTER ABSTRACTS

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43 | USING ANATOMIC LANDMARKS FOR RELIABLE CANINE BRAIN SAMPLING IN NONCLINICAL GENERAL TOXICITY STUDIES

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Introduction
Microscopic evaluation of the brain is an important component of nonclinical toxicity studies with well-established recommendations for examined neuroanatomic structures (caudate/putamen, cerebellum, cerebral cortex, choroid plexus, hippocampus, hypothalamus, medulla oblongata, midbrain, pons, and thalamus). Published sampling guides for dogs have used a matrix or extrapolation from nonhuman primates. Our goal was to develop a canine brain trimming scheme utilizing anatomic landmarks to capture recommended neuroanatomic structures for evaluation in nonclinical general toxicity studies.

Methods
Veterinary pathologists and laboratory staff drafted sampling guidelines utilizing anatomical landmarks. Coronal sections were acquired from Beagle dog brains, processed routinely, and evaluated. Anatomic landmarks were refined following iterative evaluation of sections.

Results
Consistent capture of recommended structures was achieved utilizing major landmarks: (1) olfactory tracts and piriform lobes; (2) optic chiasm; (3) infundibulum; (4) cerebral peduncles; (5) pons and cerebellum; (6) occipital lobe; and (7) cerebellar vermis and parafolliculi. Additional corroborative landmarks on respective sections included: corpus callosum and caudate nucleus; anterior commissure; amygdala; medial geniculate nucleus; colliculi, sulcus splenialis, and absence of corpus callosum; occipital white and gray matter; and cerebellar folia, cerebellar peduncles, and medulla oblongata. The seven levels were processed onto nine slides (bisecting levels 4 and 5). Use of anatomic landmarks required no special equipment and allowed for consistency across brains that varied in size.

Conclusion
Utilizing recognizable anatomic landmarks allows for consistent acquisition of sections containing recommended neuroanatomical structures for general toxicity studies. Reliable sectioning can be achieved without special equipment and across normal biologic variation in brain size.

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44 | RENAL TUBULAR PIGMENTATION IN EXPERIMENTAL BEAGLE DOGS

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Introduction
Cytoplasmic pigmentation in renal tubules is observed in various pathological conditions including lipofuscinosis, hemosiderosis, hemoglobinuria and myoglobinuria.[1,2] In experimental Beagle dogs the presence of coarsely granular brown to goldish pigment within tubular epithelium is a known incidental finding. Although pigment is generally assumed to be lipofuscin, a detailed characterization of its composition is lacking, and its true nature remains unclear.[3]

Methods
Renal samples from Beagle dogs with cortical tubular pigmentation were collected and 4 µm-thick serial sections were stained with Hematoxylin and Eosin (H&E) and with a panel of histochemical stainings including Giemsa, Perls Prussian blue, Hall's, Ziehl-Neelsen and Periodic acid-Schiff reaction. Both unstained blank sections and H&E-stained sections were, additionally, analyzed under UV light to assess pigment autofluorescence and additional samples were routinely processed for Transmission Electron Microscopy.

Results
Pigments did not fluoresce under UV light in H&E-stained slides, assumed an orange tinge in unstained slides under UV light and reacted positively to Giemsa and negatively to Ziehl-Neelsen, Hall's, Perls, and PAS. Electron microscopy revealed numerous amorphous aggregates of coarse to finely granular electron-dense membrane-bound material intermixed to round electron-dense clumps.

Conclusion
While pigment's electron microscopy appearance suggests some similarities to lipofuscins, the lack of autofluorescence and the negative reactions to PAS and Ziehl-Neelsen were unusual for typical lipofuscins. Further investigations are thus needed to fully elucidate the nature of this renal intracytoplasmic pigment in experimental Beagle dogs.

POSTER ABSTRACTS

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45 | HEPATOBILIARY TOXICITY AND DISTRIBUTION OF 10 NM SILVER NANOPARTICLES AFTER SINGLE INTRAVENOUS ADMINISTRATION IN MICE

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Introduction
Silver nanoparticles (AgNPs) are nano-sized materials ranging from 1 to 100 nm with distinctive chemical, physical, and biological properties[1]. Toxicological studies are required to foster knowledge about their environmental and health impact. Research on animal models reports toxic action of AgNPs on several organs like lungs, liver, kidneys, spleen, brain, and parotid glands, following different routes of exposure[2].
This study aimed to improve the understanding of the previously reported AgNPs-related hepatobiliary toxicity[3] by investigating their intra-hepatic distribution.

Methods
Male CD-1(ICR) mice were randomly divided in two groups (n=3) and intravenously administered with either vehicle or 10 mg/kg bw of 10 nm AgNPs. After 24 hours, liver and gallbladder were fixed, paraffin-embedded, and sectioned at 4 µm thickness for Hematoxylin and Eosin (H&E) staining. Autometallography and immunofluorescence (anti-Iba1, anti-Arginase1, anti-CD31, and anti-LYVE-1) were performed to analyze tissue distribution and cellular localization of silver. Images were acquired using a Leica TCS SP8 confocal microscope at 63x/1.4 magnification, and Z-stack images of a few microns were obtained for each field of view.

Results
The treatment induced severe hepatobiliary lesions, including hepatocellular necrosis and hemorrhage of the gall bladder wall. Silver aggregates were detected through different methods mainly in Kupffer cells, but also within hepatocytes, and endothelial cells.

Conclusion
The severe hepatobiliary toxicity induced by 10 nm AgNPs is related to their massive elimination in the bile[4]. In the affected livers, silver was identified within different cell types, indicating the ability of nanoparticles to penetrate and potentially cause cytotoxic effects in multiple cell populations.

POSTER ABSTRACTS

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46 | PATHOLOGICAL CHARACTERIZATION OF IRON DRIVEN TOXICITY IN BEAGLE DOGS IN A 7-DAY IV INFUSION STUDY

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Introduction
Iron oxide nanoparticles (IONPS) are common natural or synthesized compounds used as contrast agents in magnetic resonance imaging (MRI), effective drug delivery in the treatment of neurological disorders and cancer, and as nanoadjuvant for vaccine and antibody production.

Methods
This case describes the pathology and clinical pathology findings obtained in a 7-day IV infusion toxicity study in Beagle dogs administered on D1 and D7 a test item containing IONPS formulated with a DNA. There was 1 animal per dose per sex and the doses tested were 0, 20 or 100 mg/kg/dose. All study procedures were approved by the Institutional Animal Care and Use Committee and conducted in compliance with animal welfare regulatory authorities.

Results
Microscopic findings were mainly observed at 100 mg/kg/dose and included minimal to marked necrosis in many tissues, intracellular and extracellular pigments (correlated with dark discoloration grossly), hemorrhages (correlated with dark foci grossly and likely secondary to the clotting time prolongation observed in clinical pathology), intrasinusoidal erythrocytes in lymph nodes (correlated with mottled discoloration grossly), Kupffer cell hypertrophy/hyperplasia and oval cell hyperplasia in the liver and, decrease in lymphoid cellularity in the spleen and thymus (correlated with lower thymus weights). Pigments were positive for Perl's staining, indicating "non-heme" iron in these tissues such as ferritin and hemosiderin and were therefore considered secondary to the iron contained in the IONPS.

Conclusion
Pathology findings related to high concentration of iron intravenously administered have been rarely reported and investigated in laboratory animals and need to be further characterized in non-clinical studies as more and more innovative therapies using IONPS are emerging.

POSTER ABSTRACTS

Toxicological Pathology

47 | CHARACTERIZING CANDIDATES FOR GREATER BIOBEHAVIORAL TRANSLATIONALITY AND REFINEMENT: THE MINIPIG AND NHP HAVE SIMILAR CNS SPONTANEOUS FINDINGS (A FIRST STEP)

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Introduction
Growing neurologic and associated behavioral disorders afflict our modern society and require animal models with greater biobehavioral translationality from discovery to non-clinical safety assessment of small and large molecules. The non human primate (NHP) is considered the most translational animal model followed by other species. There is growing evidence that pigs are an important animal model with significant similarities to human neuroanatomy, physiology, and behavior.

Methods
Contemporary and retrospective assessment of brain findings of historical control minipig was performed. Type, frequency, and anatomic distribution of microscopic findings were collated and characterized by sex and age. Findings were categorized as “spontaneous” or “administration procedure related” (APR). Spontaneous findings were compared to what has been reported in NHP.

Results
Preliminary Results:From 12 animals and 6 different studies, there were 3 females and 9 males, between 2-14 weeks old with no age specified for 3 males. Administration routes were intraparenchymal (IP, N=2), Intravenous (bolus or infusion, N=2), and oral gavage (N=8). The lesions were mononuclear cell infiltrate (N=7), gliosis (N=2), necrosis (white/gray matter, N=2) and meningeal mineralization (N=1). All spontaneous findings were minimal and marked necrosis was APR (IP administration). Distribution of the lesions were focal (N=7), multifocal (N=1) or non-specified (N=4). Mononuclear cell infiltrate occurred in the meninges (N=4), choroid plexus (N=2), cerebrum (N=1) and/or pons (N=1). Gliosis was reported in the cortical cerebrum or hippocampus (N=2).

Conclusion
Minipigs and NHP have comparable CNS spontaneous findings. Characterization of minipig CNS spontaneous lesions is important in the assessment of its potential for greater biobehavioral translationality.

POSTER ABSTRACTS

Toxicological Pathology

48 | CARDIOMYOPATHY WITH FUNCTIONAL IMPAIRMENT IN MATURE CYNOMOLGUS MACAQUES

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Introduction
There is limited historical control data and literature on the range and incidence of spontaneous pathology in sexually mature macaques used in nonclinical safety testing. Small foci of myocardial degeneration are common in Mauritian macaques, but cardiomyopathy is rare and spontaneous cases with functional impairment have not been reported.

Methods
A 6-month general toxicity study was performed in sexually mature Mauritian cynomolgus macaques, aged 4.5 to 7.5 years, including ECG and blood pressure evaluation and standard pathology workup. Masson-Trichrome and Perl's Prussian blue stains were applied on selected heart and lung sections.

Results
Features of myocardial degeneration were present in 5/6 control males and 3/6 control females. Two males showed large areas of degeneration in the left ventricle, consistent with cardiomyopathy, associated with an up to 2.5-fold increase in absolute and relative heart weight compared to unaffected controls. Microscopically, the process was characterized by disarray, vacuolation, karyomegaly, and loss of cardiomyocytes, accompanied by prominent fibrosis as well as mononuclear infiltrates. Dilatation of the left ventricle and atrium, chronic pulmonary congestion, and abnormalities in ECG and blood pressure were additionally present in one male, indicating decompensation. In historical control data cardiomyopathy or myocardial degeneration were noted in 9% of macaques aged over 5 years (13/211 animals).

Conclusion
The report demonstrates an unusually high incidence and severity of spontaneous myocardial lesions in older macaques. It will support categorization of comparable lesions as background pathology and help to avoid misidentification as test-article related effects.

POSTER ABSTRACTS

Toxicological Pathology

49 | INHAND: INTERNATIONAL HARMONIZATION OF NOMENCLATURE AND DIAGNOSTIC CRITERIA FOR LESIONS-AN UPDATE-2024

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Introduction
The INHAND Proposal (International Harmonization of Nomenclature and Diagnostic Criteria for Lesions in Rats and Mice) has been operational since 2005. A Global Editorial Steering Committee (GESC) coordinates objectives of the project. Terminology for common nonclinical toxicity species is the responsibility of Working Groups, with experts from North America, Europe, and Japan. All rodent organ systems have been published in Toxicologic Pathology or in the Journal of Toxicologic Pathology as supplements and on a web site - www.goReni.org, with most recent change control and updates readily available at goRENI.org. Mini-pig and Dog were published in Toxicologic Pathology in 2021 and Non-human primate and Rabbit were published in the Journal of Toxicologic Pathology in 2021. Non-rodent Ocular working group manuscript will be submitted to Toxicologic Pathology for publication in Spring 2024 and the Fish manuscript will be ready for membership review in late 2024. INHAND guides and goRENI.org offer terminology, diagnostic criteria, differential diagnoses, images, and guidelines for recording lesions in nonclinical toxicity and carcinogenicity studies. INHAND GESC representatives work with representatives of FDA Center for Drug Evaluation and Research (CDER), Clinical Data Interchange Standards Consortium (CDISC), and National Cancer Institute (NCI) Enterprise Vocabulary Services (EVS) to incorporate INHAND terminology as preferred terminology for SEND (Standard for Exchange of Nonclinical Data) submissions to the FDA. Interest in INHAND nomenclature, based on input from industry and government scientists, is encouraging wide acceptance of this nomenclature.

Methods N.A.
Results N.A.
Conclusion N.A.

POSTER ABSTRACTS

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50 | INFLAMMATORY BOWEL DISEASE IN BEAGLE DOGS FROM TOXICITY STUDIES: HOW CAN WE EVALUATE ITS IMPACT?

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Introduction
Inflammatory Bowel Disease (IBD) is a well-known idiopathic condition that chronically affects the gastrointestinal tract. The diagnosis of this condition is established when mucosal inflammation is histologically demonstrated, and all other potential causes of gastroenteritis have been ruled out. Although IBD is a common gastrointestinal issue in dogs, there is currently no information available regarding its incidence and impact in chronic toxicity studies. The objective of this study is to describe cases of IBD in Beagle dogs within the context of toxicity studies.

Methods
A 6-month-old male dog had to be euthanized for animal welfare reasons before being included in a chronic toxicity study. Follow-up evaluations were conducted on animals from the same batch participating in two separate chronic toxicity studies. The digestive tracts of these animals were assessed both histologically and immunohistochemically.
Results
Microscopic examination of the small intestine, and to a lesser extent, the large intestine, revealed severe chronic enteritis characterized by a diffuse infiltrate of eosinophils, plasma cells, and lymphocytes in the mucosa and submucosa. Additionally, multifocal necrosis affected the mucosa. In regions without necrosis, the villi were markedly shortened and blunted, often accompanied by dilated lymphatic vessels.

Conclusion
Cases of IBD in Beagle dogs within the context of chronic toxicity studies are infrequently diagnosed and can sometimes be misidentified due to histological findings related to the test article. This study contributes to a better understanding of this disease in young Beagle dogs, emphasizing the importance of diagnosing IBD during toxicity evaluations and assessing potential impacts during in vivo preclinical phase.

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52 | INNOBALM® - AN ALTERNATIVE TO FORMALIN FIXATION

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Introduction
Fixation is an essential step in preservation of cadavers and tissues for histological processing. Formalin does have its critics because of the safety risks. Innobalm® Histology Fixation Solution contains Aminolipin® a patented substance and is currently being tested as a possible alternative to formalin fixation. In contrast to formaldehyde, Aminolipin® denatures the proteins by unfolding the three-dimensional structure without cross-linking of the protein side chains. An extensive comparative study was performed at AnaPath Services GmbH and AnaPath Research S.A.U. The scientific aim was to investigate the advantages and disadvantages of a new alternative to formalin fixation. There is no conflict of interest in the research about this product.

Methods
Rat tissues were used for comparison. Perfusion and immersion fixation was performed using Innobalm® Histology Fixation Solutions in comparison to neutral phosphate-buffered 10% formalin and 2% glutaraldehyde. Selected tissues (brain, kidney, liver, pancreas, spleen, lung, ileum) were evaluated by routine histology (hematoxylin-eosin, Masson's trichrome, Gömori, PAS) and immunohistochemistry (Brain: GFAP, NF200; Kidney: CK20, ASMA, Col I, CD31; Spleen: CD3, CD20.

Results
Perfusion fixation with Aminolipin®-containing solutions (Innobalm®) resulted in better color preservation of the organs than with formalin and glutaraldehyde. In addition, the carcasses and tissues remained soft and flexible when prepared with Innobalm®. Histologically, routine stains and immunohistochemistry revealed high quality comparable results.

Conclusion
In contrast to formaldehyde, Aminolipin® denatures the proteins by unfolding the three-dimensional structure without cross-linking of the protein side chains. Based on its mechanism and the evidence provided by our results, Innobalm® containing the novel substance Aminolipin® is a reliable fixation alternative when aiming for high quality tissue evaluation.

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57 | A NOVEL DIGITAL ANALYSIS FOR THE EVALUATION OF MICROGLIA CELLS IN THE INJURED BRAIN

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Introduction
Emerging evidence indicates that microglia are playing a major role in pathological and healing processes in the brain, expressing different morphological phenotype. Immunohistochemistry (IHC) enables labeling these cells and differentiation between their sub-types, yet an accurate analysis of their morphological changes in relation to their physiological activity is still lacking. The present study demonstrates a novel digital analysis for measurement of various morphological parameters in microglia at distinct phases in the ischemic brain.

Methods
IHC of IBA1 was used for the labelling of microglia in paraffin brain sections of naïve and ischemic rats. Digital analysis of soma and bifurcations was performed in the penumbra, the transition region between the infarct and the intact tissue, using a multi-layer perception neural network (MLP-NN), based on branches' morphology. A similar analysis was conducted with CD16 and CD206 markers for M1 and M2 microglia sub-types.

Results
Histological evaluation of the ischemic brain revealed an increase of activated IBA1 positive cells, displaying ameboid body with retracted branches, compared to low number of microglia in the naive brain, displaying normal ramified morphology. Digital analysis of the structural characteristics demonstrated a relationship between lesion severity and the number of branch bifurcations and endpoints. A parallel pattern was found for the pro and anti-inflammatory M1 and M2 cells.

Conclusion
The ischemic lesion is accompanied by activation of specific microglia phenotypes, in parallel with the pathological changes. The present study demonstrates the use of machine learning and advanced tools for the quantitation of varied morphological changes in relation to their functions.

POSTER ABSTRACTS

Veterinary Pathology: Livestock

32 | LIMOSILACTOBACILLUS REUTERI B1/1 ENHANCED LOCAL IMMUNE REACTION TO SALMONELLA ENTERITIDIS PT4 ON CHICKEN ILEAL EXPLANT CULTURE

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Background
Poultry consumption is becoming increasingly popular worldwide. EFSA reports that common foodborne infections in the EU are mainly caused by Salmonella strains from poultry products, which pose a direct threat to the consumer. The use of antibiotics is prohibited, which opens up space for the use of probiotic bacteria such as Limosilactobacillus strain in the prevention and treatment of these diseases. To test their action, it is possible to use tissue biopsies (explants), which realistically mimic the in vivo situation and host-pathogen interactions.

Materials & Methods
The experiment was divided in to 4 groups on prepared chicken ileal explants (96 well plate): control, L. reuteri B1/1 (LR) (10^9 CFU), S. enteritidis PT4 (10^7 CFU) and pre-treatment (LR for 2 h; washing; SE PT4 for another 2 h), each incubated: 37°C; 5% CO_2, then were washed and collected for Real-Time PCR (relative expression for cytokines: IL-8, IL-15, TNFα, TGFβ4) and ELISA (alpha 1 acid glycoprotein-AGP) analysis.

Results
We recorded an enhancement of the local inflammatory response through the upregulation of relative gene expression for all pro-inflammatory cytokines in the pre-treatment group compared to other group, which correlates with increased production of AGP (pg/ml) in ileal explants ($P<0.05$; $P<0.01$, $P<0.001$, $P<0.0001$). On the contrary, the relative gene expression for TGFβ4 was down-regulated in both infected groups.

Conclusion
The immunomodulatory effect of L. reuteri B1/1 was observed in the early phase of S. enteritidis PT4 infection. Moreover, new ex vivo ileal explant model proves to be suitable candidate for study of pathological changes in the intestine of animals.

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Veterinary Pathology: Livestock

34 | BACILLUS AMYLOLIQUEFACIENS CECT 5940 IMPROVED MUCOSAL LOCAL IMMUNITY IN THE GUT OF BROILER CHICKENS

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Background
Lactic acid bacteria as natural substances are also used in the veterinary field in various forms of application. Bacillus amyloliquefaciens (the main component of ECOBIOL) is a facultative anaerobic bacterium with the ability to secrete many enzymes, which can improve the production parameters of broilers. In terms of testing the complex action of ECOBIOL preparation, it is necessary to focus on monitoring its effect on local mucosal immunity in the intestine of broiler chickens.

Materials & Methods
60 one day old broiler chickens (ROSS 308) were divided into 2 groups (n= 30): control and probiotic group - chicks were sprayed with ECOBIOL (Evonik) during first 5 days and in the second phase they received it in drinking water. The experiment lasted 11 days. Samples of the jejunum and ileum were taken to determine gene expression (Muc-2, IgA, E-cadherin, occludin, claudin-1) in RNA later. The level of relative gene expression was determined using the quantitative Real-Time PCR method.

Results
The application of B. amyloliquefaciens increased the level of relative gene expression for selected parameters (Muc-2, IgA, E-cadherin, occludin, claudin-1) in both part of chicken intestine compared to control group ($P < 0.05$, $P < 0.01$, $P < 0.001$).

Conclusion
The application of Bacillus amyloliquefaciens CECT 5940 had a beneficial effect on gene expression for selected parameters in the intestine of chickens. Therefore, we assume that the application of B. amyloliquefaciens may improve the local mucosal immunity of broiler chickens.

POSTER ABSTRACTS

Veterinary Pathology: Livestock

57 | PORCINE RESPIRATORY DISEASE COMPLEX: LINKING OLD AND EMERGING VIRUSES ON SWINE FARMS

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Background
Porcine Respiratory Disease Complex (PRDC) causes economic losses in the swine industry due to viral, bacterial, and environmental factors. In contrast to known viruses (PRRSV, PCV2), emerging viruses such as PCV3, PPV2, and TTSuV are less studied. This study investigates lung lesions in different swine age groups in association with both historically reported (PRRSV and PCV2) and emerging viruses (PCV3, PPV2, TTSuV).

Materials & Methods
Lung tissue samples from 10 adult pigs (AP), 10 post-weaning pigs (PW), and 9 piglets (PG) underwent macroscopic and histological examination. RT-PCR detected PRRSV, PCV2, PCV3, and TTSuV genomes. Sanger sequencing confirmed the specificity of RT-PCR for PPV2 and TTSuV.

Results
PRRS was found in 33% of piglets, 20% of adults and PW pigs, with low viral loads. PCV2 was present in all adult pigs, 33% of PW, and 22% of PG. PCV3 was detected mainly in piglets (44%), followed by PW (20%) and A (10%). TTSuV was found in all groups, with higher loads in adults than PW pigs and PG. PPV2 was found in all adults and 90% of piglet lungs, and less in PW pigs (50%). Sequencing revealed 97% similarity between PPV2 and Chinese strains while TTSuV1 and TTSuV2k showed similarity to strains from Brazil and South Korea. Histologically, infected animals showed moderate, diffuse chronic lymphoplasmacytic interstitial pneumonia in 60% of adults, 100% of post-weaning pigs, and 100% of piglets.

Conclusion
The results highlight the need for comprehensive strategies for PRDC management, targeting both historically reported and emerging viruses.

POSTER ABSTRACTS

Veterinary Pathology: Livestock

86 | RUMEN HISTOLOGY REVEALED: MAPPING THE MICROSCOPIC TERRAIN OF SUBTLE MORPHOLOGICAL CHANGES

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Background
There are no descriptions of subtle pathological changes that may potentially indicate abnormal ruminal structure and function. This study aimed to describe all the microscopic changes that may be observed at post-mortem examination, and propose a scoring system for a standardised histological assessment of the rumen.

Materials & Methods
Forty-four rumen samples were collected from healthy cattle at the slaughterhouse and assessed for papillae colour and shape, ventral sac gross features and presence of lesions using a A-F scoring system as per Jonsson et al. (2020). Histological sections were scored based on the distribution of lesions (3-tier scoring; mild to severe) with the final parakeratotic score obtained by multiplying the severity score by the distribution score (% of papillae affected).

Results
Grossly, 9/44 samples (20.5%) samples had yellow discoloured papillae, 2/44 (4.5%) abnormal papillae shape (short/thin/oval-shaped) and 4/44 (9%) areas devoid of papillae or damaged. Microscopically, all the samples showed parakeratosis and acanthosis with a score of 2-3 (moderate-high) in 21/43 (49%) and 41/43 (96%) of the samples, respectively. Dyskeratosis, hydropic degeneration and basophilic/mucinous epithelial cytoplasm were observed in 34/43 (79.1%), 41/43 (95.3%) and 20/43 (46.5%) of the samples, respectively. Mild to moderate degree of intraepithelial vesicles formation, surface mineralisation and sloughing of the stratum corneum were present in 10/43 (23.2%), 26/43 (60.4%) and 18/43 (41.9%) of the cases, respectively.

Conclusion
Although not clinically or grossly relevant, these microscopic changes may suggest a common subacute-chronic rumen damage and should be carefully evaluated, especially if affecting large areas of the rumen.

POSTER ABSTRACTS

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106 | EVALUATION OF LESIONS COMPATIBLE WITH CAPRINE PARATUBERCULOSIS IN SLAUGHTERHOUSE ON THE ISLAND OF GRAN CANARIA, SPAIN

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Background
Paratuberculosis (PTB) is a chronic disease caused by Mycobacterium avium subsp. paratuberculosis. The prevalence of the disease in Gran Canaria is unknown, and the anatomopathological examination in the slaughterhouse is an important tool for its diagnosis. The purpose of the study was to evaluate lesions compatible with PTB in goats at the slaughterhouse in Gran Canaria.

Materials & Methods
According to the goats slaughtered in 2023, 91 animals were statistically representative to be evaluated using a sample size calculator. The mesenteric and ileocecal lymph nodes (MS LNs; IC LNs) and ileocecal valves (ICV) with or without macroscopic lesions were selected. The samples were processed for histological analysis using routine HE technique. Statistical analysis was performed using the Shapiro-Wilk, Mann-Whitney U, Kruskall-Wallis and Chi-square tests with IBM SPSS Statistics 28.

Results
The 91 animals (20 males and 71 females) belonged to 17 farms from 12 of Gran Canaria's 21 municipalities. The age and weight had a median of 34 months and 21kg. Granulomatous lymphadenitis in MS LNs and ICV mucosal thickening were macroscopically identified in 18.7% and 20.9%, respectively, in 47% of the farms analyzed. Histologically, granulomatous lesions increased by 41.75% of LNs, improving the diagnosis by 123%. In VIC, lesions were only identified microscopically in 15.88%. No significant differences between the macroscopic and microscopic lesions were observed regarding sex, age and carcass weight.

Conclusion
This cross-sectional study contributes to understanding the problem of goat PTB in Gran Canaria Island, highlighting pathological anatomy in the slaughterhouse as a surveillance tool.

POSTER ABSTRACTS

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146 | KERATIN AND SQUAMOUS EPITHELIA: A NOVEL FINDING WITHIN SUBCUTANEOUS POST-VACCINATION GRANULOMAS IN SHEEP

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Background
Vaccination is one of the most useful tools to prevent and control infectious diseases. Health programs in sheep can include multiple vaccine injections in one single animal per year depending on several factors. Granulomas following subcutaneous administration of aluminum-based vaccines in sheep are constant reactions mainly composed by activated macrophages and multinucleated giant cells. In other species, these chronic inflammatory reactions have been associated with the development of follicular cysts or neoplasias as pilomatricomas. The aim of this study is to describe keratin and squamous epithelia as novel microscopic findings in aluminum-induced granulomas in sheep.

Materials & Methods
Seventeen lambs were subjected to an inoculation protocol that included two commercial AlOOH-containing vaccines and their booster. Protocol was repeated 5 months later (8 injections in total). Pathological features were evaluated on 112 post-vaccination granulomas.

Results
Eight out of 17 (47.1%) lambs showed at least one granuloma with layers of orthokeratotic keratin, commonly lined by stratified squamous epithelium between the inflammatory reaction and the necrotic content. In addition, a small follicular-like cyst was observed in one granuloma. These findings were only found in granulomas 2-3 months old but not in those 7-8 months of age.

Conclusion
This is the first description of keratin and squamous epithelia within post-vaccination granulomas in sheep, elements that seem to be mostly removed by the inflammatory reaction. However, in some cases they might persist and evolve into follicular cysts, a skin condition frequently observed in the subcutaneous tissue of sheep.

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197 | COMPARATIVE NEUROPATHOLOGICAL STUDY OF SHEEP INOCULATED WITH A NATURAL AND A RECOMBINANT STRAIN OF SCRAPIE PRIONS

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Background
Scrapie is a prion disease affecting sheep, characterized by the accumulation of PrPSc (prion protein) deposits primarily in the central nervous system (CNS). Generation of prion disease by inoculation of exogenously produced recombinant prions is considered the ultimate proof of the prion hypothesis. However, this is still controversial. This study focuses on the neuropathological characterization of sheep inoculated with a recombinant strain of classical scrapie, comparing it to a natural strain.

Materials & Methods
Six Rasa Aragonesa sheep infected with scrapie were selected: two with a natural strain (VRQ/VRQ and ARQ/ARQ PRNP genotypes) and four with a recombinant prion strain (2 VRQ/VRQ, 2 ARQ/ARQ). Sheep were euthanized, and tissue samples from the lymphoid and CNS were analyzed using immunohistochemistry and haematoxylin-eosin staining to compare the prion lesions.

Results
The same neuropathological lesions were observed in VRQ/VRQ sheep inoculated with either the natural or recombinant scrapie strain. These included spongiosis of the neuropil, intraneuronal vacuolization, and abundant PrPSc deposition both in the CNS and lymphoid tissues. The distribution pattern in the CNS and lymphoid tissues was very similar between these animals. However, no spongiform lesions or PrPSc accumulation were detected in ARQ/ARQ sheep inoculated with the recombinant strain, unlike ARQ/ARQ sheep infected with the natural strain, which showed similar lesions to VRQ/VRQ sheep.

Conclusion
No significant differences were observed in the distribution of prions in the lymphoid and nervous systems of VRQ/VRQ sheep inoculated with either the recombinant or natural strain. However, the synthetic strain could not reproduce the disease in ARQ/ARQ sheep.

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210 | ICHTHYOSIS IN A STILLBORN TEXEL-CROSSBRED LAMB

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Background
Ichthyosis is a group of conditions resulting in generalised hyperkeratosis and alopecia. In animal species, the condition is best described in cattle and dogs, in which it is often thought to have a recessive mode of inheritance. However, it is exceedingly rare in sheep, with only one other report in the literature. We present a case of ichthyosis in a stillborn Texel-crossbred lamb.

Materials & Methods
Gross and microscopic pathological examination of the skin was conducted.

Results
There was generalised thickening and scaling of the skin, which was particularly severe over the face and limbs. Microscopic examination of samples of skin revealed marked thickening of the stratum corneum, with compact layers of keratin entrapping hair shafts (orthokeratotic hyperkeratosis) and concentric layers of keratin accumulating within hair follicle infundibula (follicular keratosis). There was also ballooning degeneration of cells in the strata spinosum and granulosum, and coarse and irregular keratohyaline granules. The microscopic appearance of the skin in this case was consistent with the epidermolytic form of ichthyosis, whilst the clinical presentation resembled the lethal congenital form in cattle (ichthyosis fetalis). This was a single case in the flock and the genetic basis and mode of inheritance for the condition was not determined.

Conclusion
Ichthyosis is an exceedingly rare condition in sheep but should be considered in lambs is generalised thickening and scaling of the skin.

POSTER ABSTRACTS

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236 | CHARACTERIZATION OF LUNG AND THYMUS LESIONS AS TARGET ORGANS IN PIGLETS INFECTED WITH A NEW VIRULENT PRRSV-1 STRAIN

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Background
In 2020, a new virulent porcine reproductive and respiratory syndrome virus (PRRSV) strain, named Rosalía, emerged in Spain associated with high morbidity, and mortality in piglets. The aim of this study was to evaluate the macroscopic and microscopic lesions in the lung and thymus, as target organs, during early and advanced stages of infection with Rosalía in comparison with the classic 3249 PRRSV-1 strain.

Materials & Methods
Fifty-four 4-week-old pigs were assigned into 3 groups, one control and two infected with either 3249 or Rosalía strain. At 7, 14 and 42 dpi, 6 animals/group were euthanized to evaluate gross lesions and samples from the lung and thymus were collected for histopathological study. Weight, clinical signs, and temperature were recorded along the study.

Results
Pulmonary gross lesions were more intense in both infected groups at 7 dpi, with a decrease in thymus consistency, specially marked in Rosalía-infected animals. At 42 dpi, lung lesion score remained high only for Rosalía group compared to 3249 and control groups. Microscopically, animals from both infected groups exhibited interstitial pneumonia accompanied by areas of suppurative bronchopneumonia and proliferative necrotizing pneumonia only in Rosalía-infected pigs. Animals from this group also displayed severe cortical thymic atrophy and stroma proliferation, whereas a “starry sky” appearance was observed in less affected animals from both infected groups. Eventually, these lesions decreased in intensity at 42 dpi.

Conclusion
Macroscopic and histopathological evaluation showed that Rosalía strain induces more severe lesions in lung and thymus, particularly in the early stage of infection.

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237 | LUNG LESION CHARACTERISATION OF A HIGHLY VIRULENT STRAIN OF PRRSV-1 CIRCULATING IN SPAIN SINCE 2020

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Background

In 2020, Spain experienced outbreaks of unprecedented severity caused by a novel recombinant strain of porcine reproductive and respiratory syndrome virus (PRRSV) known as Rosalia. This study aims to evaluate the impact of Rosalía strain on the lung of piglets infected experimentally at 10- and 35-days post infection (dpi).

Materials & Methods

Twenty-four piglets were intranasally inoculated with the virulent Rosalia PRRSV-1 strain (n=12) or mock inoculated (n=12), then euthanised at 10 and 35 dpi. Gross lung lesions were recorded at necropsy based on pneumonic lung proportion in each lobe. Lung samples were collected for histopathological and immunohistochemical studies against CD3, CD20, calprotectin and factor VIII. Sera were collected to evaluate viremia dynamics (RT-qPCR).

Results

After challenge, pigs developed viremia, persisting in most animals until 35 dpi. Piglets infected with Rosalia strain exhibited severe interstitial pneumonia, featuring several bronchopneumonia areas, and progressing to proliferative necrotising pneumonia in several animals, particularly evident at 35 dpi. Alveolar septa thickening was marked, with increased calprotectin$^+$ cells at 10 dpi and predominantly CD20$^+$ cells at 35 dpi. Perivascular and peribronchial infiltrates were mainly characterised by mononuclear cells, primarily CD3$^+$ at 10 dpi and CD20$^+$ at 35 dpi, compared to control group. Endothelial activation was observed in association with perivascular infiltrates at time points. Notably, tertiary lymphoid organs-like structures, mainly CD20$^+$ cells, were found at 35 dpi.

Conclusion

Compared with classical PRRSV strain, Rosalia induced severe interstitial pneumonia progressing to a proliferative necrotising pneumonia in several animals. Endothelial cell activation occurred in regions with perivascular mononuclear infiltrates in infected piglets.

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256 | CHARACTERIZATION OF PULMONARY CELL INFILTRATION AT THE EARLY STAGE OF INFECTION WITH CLASSIC AND VIRULENT PRRSV-1 STRAINS

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Background
In recent years, virulent strains of porcine reproductive and respiratory syndrome virus (PRRSV) have emerged, causing intensified clinical signs and lesions. This study aims to compare and characterize lung lesions induced by PRRSV-1 strains of different virulence.

Materials & Methods
A retrospective study was conducted using lung samples from piglets inoculated with classic (LV, 215-06, 3249) or virulent (SU1-Bel, Lena, Rosalia) PRRSV-1 strains and euthanized at 7-10 dpi. Histopathological lesions and the immunohistochemical expression of CD3, CD20, calprotectin, CD14, CD163, and TTF-1 were assessed.

Results
Piglets inoculated with virulent PRRSV-1 strains displayed a more intense interstitial pneumonia together with perivascular and peribronchiolar cell infiltrate and areas of suppurative bronchopneumonia in the case of Lena and Rosalía. Interstitial pneumonia was defined by an infiltration of $CD3^+$ T-cells, which was significantly higher for 3249, Lena and Rosalía strains, as well as infiltration of calprotectin$^+$ (myeloid) cells in the alveolar septa (without differences among strains). In a lesser extent, alveolar septa also contained $CD20^+$ B-cells, showing a significant increase in Rosalía-infected piglets. A decreasing trend in the frequency of $CD163^+$ cells parallel to an increase in $CD14^+$ (monocytes/macrophages) and TTF-1^+ cells (type 2 pneumocytes) was observed according to the virulence of the strain.

Conclusion
T cells, myeloid cells, and in a lesser extent B-cells characterized the interstitial pneumonia induced by PRRSV-1 strains at the early stages of infection, together with type 2 pneumocytes and a decrease in $CD163^+$ cells, changes that trend to be more intense with virulent strains.

POSTER ABSTRACTS

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258 | EFFECT OF MILK THISTLE (SILYBUM MARIANUM) EXTRACT ON PRODUCTIVITY AND HEPATOPROTECTION IN COMMERCIAL LAYING HENS: A COMPREHENSIVE EVALUATION

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Background
Milk thistle (Silybum marianum) extract, known for its antioxidant and hepatoprotective properties, is often added to animal nutrition. Considering its documented benefits in various animal studies, we hypothesized that it would also be crucial to investigate its role in the diet of laying hens prone to fatty liver-hemorrhagic syndrome (FLHS).

Materials & Methods
Sixty Isa-brown laying hens in late production stage were randomly allocated into four treatment groups (T1 to T4), each receiving diet with different levels of milk thistle extract (T1: 0%, T2: 1%, T3: 2.5%, and T4: 4%) for 8 weeks. Egg quality parameters, yolk lipid oxidation, total phenolic content (TPC), and fatty acid profile were assessed. Individual hen performance and their liver malondialdehyde (MDA) content were determined. Liver specimens were examined grossly and histologically to evaluate hepatocellular lesions such as vacuolization (lipidosis), reticular stromal architecture, the amount of collagenous connective tissue, and vascular wall changes using HE, Masson's trichrome, Weigert-Van Gieson, and Silver stains, as well as TNF-α specific immunohistochemistry.

Results
No significant differences were observed regarding egg quality parameters. The T2 group had the lowest MDA values and displayed improved gross appearance with lower degrees of hepatic vacuolization compared to other groups. Liver discoloration was milder in T3 (43.8%) compared to the T1 and T4 groups (18.8% and 12.5% respectively, $P=0.013$). Reticulin loss was correlated with the degree of hepatic vacuolization ($r=0.751$, $P<0.001$).

Conclusion
Our findings suggest that supplementation of milk thistle extract at specific levels can result in beneficial hepatoprotective effects in laying hens.

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265 | INTRACELLULAR IN VIVO MICROCRYSTALLIZATION OF ALUMINIUM OXYHYDROXIDE ADJUVANT IN POST-VACCINE GRANULOMAS

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Background
Amorphous aluminium (Al) oxyhydroxide (AlOOH) adjuvant is included in vaccines as aggregates of nanoparticles that are engulfed by granuloma-forming macrophages, finally accumulating as cytoplasmic granules. Formation of large, ~50μm microcrystalloid bodies have been reported in sheep and humans, mainly intra but also extracellularly. These bodies might represent an unusual form of in vivo crystallization, a process never reported before for aluminium. The aim of this work is to characterize this crystal formation and to present fast histochemical stains, useful for Al routine diagnostic work.

Materials & Methods
Lambs (n=6) were inoculated subcutaneously with a vaccine prototype containing only AlOOH (6 mg/ml) and inactivated bluetongue virus. Granulomas were sampled at 133 days. Histopathologic studies included size and aspect ratio analysis of crystalloid bodies and different, previously-described Al specific histochemical stains. A physicochemical characterization was performed on the adjuvant stock solution.

Results
Microcrystalloid bodies showed a hexagonal to rhomboid rod-shaped morphology (mean: 42.5 μm length, 19.7 μm width), being non-birefringent, non-refractile, eosinophilic and PAS+. Modified hematoxylin stain for Al and lumogallion were the most optimal and concordant stains. Results suggest the in vivo formation of well-crystallized AlOOH (boehmite). This can occur in vitro under pH 4-5 or within amorphous AlOOH adjuvant-containing phagolysosomes, as they show a similar pH. Microcrystals were not found in the adjuvant stock solution.

Conclusion
Microcrystalloid bodies may result from crystallization of adjuvant aggregates within macrophage phagolysosomes. Modified hematoxylin stain could be useful on Al detection in tissues when lumogallion or fluorescence microscopy is not available.

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268 | ABDOMINAL CHONDROSARCOMA IN A YOUNG SHEEP

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Background
Chondrosarcomas are malignant mesenchymal neoplasms in which the neoplastic cells produce cartilaginous matrix but not osteoid or bone. This work describes a case of an abdominal chondrosarcoma in a young sheep.

Materials & Methods
A 10-month-old female sheep with history of lethargy, dyspnoea and mild fever was submitted for pathological investigations. Necropsy, histology, and immunohistochemistry according to standard procedures on selected tissues were performed.

Results
Macroscopically, abdomen was expanded by a large discrete, encapsulated, nodular, necrotic, and fleshy mass (30x22x25cm; 5.6 Kg) connected to the right caudal ribs, adherent to the parietal peritoneum, and covered by mesentery. The cut surface was polycystic pink-to-brown with multifocal to coalescing areas of liquefaction. Histologically, the lesion was multifocally necrotic and cystic, composed of moderate number of spindle cells arranged in bundles intersecting perpendicularly associated with moderate fibrovascular stroma and scattered small variably sized chondroid foci. Approximately 80% of neoplastic cells exhibited moderate to strong cytoplasmic labelling for vimentin, and a smaller proportion (5-10%) showed strong cytoplasmic labelling for S100. Dispersed amongst neoplastic cells and mostly in proximity of necrotic foci, αSMA positive elongated cells (interpreted as infiltrating stromal myofibroblasts) were present. Calponin and desmin were diffusely negative. Alcian-PAS showed a delicate mucinous matrix throughout the mass.

Conclusion
Based on the cell morphology, immunoreactivity, and the presence of areas of chondroid differentiation, a diagnosis of poorly differentiated chondrosarcoma was made.
The poor differentiation of the tumour together with the scarcity of chondroid foci through the mass made the diagnosis challenging in this case.

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275 | ADENOMATOUS POLYPOSIS IN A COW ASSOCIATED TO MUTATIONS IN THE ADENOMATOUS POLYPOSIS COLI (APC) TUMOR-SUPPRESSOR GENE

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Background
Intestinal adenomatous polyps are benign epithelial proliferations. They are common tumors in humans and may occur spontaneously or as part of a familial disease caused by mutations in the adenomatous polyposis coli (APC) tumor-suppressor gene. Intestinal adenomatous polyps are relatively uncommon in animals. Adenomatous polyposis has not been previously reported in cattle.

Materials & Methods
A 3-year-old Friesian cow with a history of weight loss, chronic diarrhea and multiple polypoid nodules in the rectum was euthanized and necropsied. Representative tissue samples of the main organs were fixed in 10% neutral buffered formalin and processed for histopathological study. Intestinal tissue was frozen (-80°C) for molecular and genetic analysis.

Results
Grossly, the mucosa of the intestine, from the duodenum to the rectum, had numerous multifocal, polypoid nodules. No other relevant macroscopic lesions were observed. Histologically, these nodules were characterized by well-demarcated, exophytic mucosal growths comprising irregular, branching tubule-glandular structures lined by tall cuboidal to columnar intestinal epithelial cells. Goblet cells were reduced across the ileum, cecum and large intestine. Intestine samples were negative for bovine papillomavirus. Whole genome sequencing was performed and three germline mutations in the APC gene, N1554D, N2418S and L2506V were identified.

Conclusion
The pathologic findings and genetic mutations identified in this case are analogous to those seen in familial forms of adenomatous polyposis in humans. Except for dogs, naturally occurring adenomatous polyposis is exceedingly rare. To the authors' knowledge, this is the first report of adenomatous polyposis associated with APC-mutations in bovines.

POSTER ABSTRACTS

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280 | FATAL RESPIRATORY DISEASE AND SEPTICAEMIA IN A CALF DUE MANNHEIMIA CAVIAE: FIRST REPORT

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Background
Mannheimia haemolytica is a commensal organism in healthy upper respiratory tract which, consequently to stress, can be a primary pathogen causing pneumonia and septicaemia with high mortality in animals and humans. In contrast, little is known regarding the significance and pathogenicity of species different than M. haemolytica.
This case describes a fatal respiratory disease and septicaemia in a calf due Mannheimia caviae.

Materials & Methods
A healthy 12-day-old calf developed sudden acute recumbency, respiratory distress and death and was submitted for post-mortem investigation. Selected tissues underwent bacteriological culture based on standard laboratory protocols and using MALDI-TOF MS according to the manufacturer's instructions, viral and bacterial PCR testing for respiratory ruminant diseases, and histological examination.

Results
Necropsy revealed moderate petechiae within conjunctiva, small and large intestinal walls, urinary bladder, splenic capsule, diffuse moderate congestion of liver, kidneys, spleen and meninges, and severe abomasal oedema. Lungs showed bilateral moderate pleural thickening of the caudal lobes and scattered multifocal red-brown foci (around 2x1x2cm) throughout the parenchyma. Histologically, multifocal acute fibrinous interstitial pneumonia associated with intravascular bacteria and thrombosis in alveolar walls were seen, with similar bacterial aggregates in the liver and spleen with adjacent necrosis suggestive of septicaemia. Laboratory tests confirmed pure growth of Mannheimia caviae from lung, liver, and brain. M. haemolytica PCR was negative.

Conclusion
M. caviaewas first described from purulent conjunctivitis in guinea pigs. To the authors' knowledge, this is the first report of fatal pneumonia and septicaemia due M. caviae being described in bovine.

POSTER ABSTRACTS

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288 | INFLUENCE OF VACCINATION ON THE PATHOGENESIS OF EXPERIMENTAL OVINE TOXOPLASMOSIS

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Background
Toxoplasmosis is a zoonosis caused by the abortifacient parasite Toxoplasma gondii, which poses a major welfare and economic challenge to the ovine industry. This study investigates the effect of the vaccine of Toxovax® (MSD Animal Health) on its pathogenesis.

Materials & Methods
Thirty-eight pregnant ewes were distributed into different groups: vaccinated and infected (G1, n=13), vaccinated and not infected (G2, n=6), not vaccinated and infected, (G3, n=13) and not vaccinated nor infected (G4, n=6). Clinical events were daily registered. Half of the animals from each group were culled 28 days post-infection (dpi). Tissue samples from foetuses, lambs and sheep were collected for histological and molecular studies.

Results
All G3 animals and 30% of G1 showed fever. Foetal death at 28 dpi occurred in 33% of G3, whereas the remaining sheep showed 1 classical and 2 early abortions, 2 stillbirths and 2 weak born lambs. Placental vascular lesions, thrombosis and necrosis and non-purulent multifocal inflammation at foetal samples were more frequently found in G3 than in G1. G3 placentomes, foetal and lamb samples harboured more T. gondii-DNA than G1. However, the parasite burden in placentomes was higher in G1 than G3. No abortions, parasite or lesions were detected in G2 nor G4.

Conclusion
Vaccination effectively protects sheep from abortion and reduces the amount of histological lesions and clinical signs in the course of toxoplasmosis, but it does not fully prevent vertical transmission. Further studies are needed to understand the immune response and the protective mechanisms of vaccination.

POSTER ABSTRACTS

Veterinary Pathology: Small Animal

11 | AUTOMATED ASSESSMENT OF KI-67 PROLIFERATION INDEX OF CANINE CUTANEOUS MAST TUMOURS USING DEEP LEARNING

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Background
Canine mast cell tumours (CMCTs) show significant behavioural variability. Manual Ki-67 nuclear immunohistochemical (IHC) scoring (Ki-67LI) has been shown to correlate with patient outcome. However, it can be laborious and lack repeatability among observers. This study validated convolutional neural networks (CNNs) for consistent and rapid Ki-67LI assessment in CMCT whole slide images (WSI), and Ki-67's prognostic significance in dermal and subcutaneous tumours.

Materials & Methods
To train and validate the algorithm, 56 CMCTs from archival FFPE biopsies, were selected. IHC staining was performed using anti-Ki-67 antibody (MIB-1). The algorithm was trained (41 WSIs) and validated (15 WSIs) to identify Ki-67-positive nuclei. For the outcome study, 180 dogs, with prior CMCTs, were selected, and their FFPE samples and clinicopathological data were collected. Ki-67LIs were calculated using the algorithm (average positive cell count per 0.0625mm^2 grid square). Univariable Cox regression established receiver operating characteristic (ROC) cut-off values.

Results
In the cohort, 18 died due to their MCT; 20 had recurrence of their MCT; 12 dogs had recurrence of their MCT and died due to their MCT. The ROC cut off value of 26.3 demonstrated significant correlation (‹ p 0.001) between Ki-67LI, with both survival and recurrence of cutaneous and subcutaneous tumours, with a sensitivity of 0.89 and specificity of 0.80.

Conclusion
The algorithm produced comparable results with published manual Ki-67LI quantification with high accuracy and efficiency, confirming Ki-67LI's strong correlation with patient outcomes. This demonstrates that CNNs can improve the accuracy and workflow efficiency of Ki-67LI assessment in CMCTs.

POSTER ABSTRACTS

Veterinary Pathology: Small Animal

16 | DEEP LEARNING-BASED ATYPICAL MITOTIC FIGURE COUNT PREDICTS SURVIVAL IN CANINE CUTANEOUS MAST CELL TUMOURS

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Background
The mitotic count (MC) is a well-established prognostic test for canine cutaneous mast cell tumours (ccMCT). While the MC includes all mitotic figures (MF), a subset of MF exhibit atypical morphologies indicating errors in chromosome segregation that lead to aneuploidy. A recent study demonstrated the prognostic relevance of the atypical MF count (AMC) in ccMCT, characterised by a high precision of 90.9% (threshold: ≥3) regarding tumour-specific survival as compared to the MC (62.5%; threshold: ›5).

Materials & Methods
The same population from the aforementioned study was used (96 ccMCT). In addition to the original pathologists (P1), a newly developed deep learning-based algorithm and an additional pathologist (P2) determined the MC and AMC. The algorithm comprised a MF detector network (YOLOR-D6) and two classifier networks (DenseNet201, EfficientNetB4) for MF subtyping. The models were trained with ›100,000 annotated and partially subtyped MF, sourced from tumour types (including ccMCT).

Results
The newly determined counts were similar to the original MCs ($r_{Algorithm}$=0.988, r_{P2}=0.952) and AMCs ($r_{Algorithm}$=0.897, r_{P2}=0.877) as determined by Pearson's correlation. Prediction of tumour-specific survival was good for all evaluators as determined by the area under the curve (AUC) for the MC (AUC_{P1}=0.885, $AUC_{Algorithm}$=0.896, AUC_{P2}=0.870) and AMC (AUC_{P1}=0.859, $AUC_{Algorithm}$=0.860, and AUC_{P2}=0.822). The high precision of the AMC (threshold: ≥3) was retained for the algorithm (77.8%) and P2 (60.0%) as compared to the evaluators' MCs.

Conclusion
This study represents the first AI-based reproduction of prognostic value for the AMC. The algorithmic predictions allow an accurate prognostic interpretation comparable to the pathologists' counts.

POSTER ABSTRACTS

Veterinary Pathology: Small Animal

28 | NAVIGATING THE DIAGNOSTIC COMPLEXITY OF SARCOMATOID RENAL CELL CARCINOMA (SRCC): A FELINE CASE REPORT

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Background
The definition of sarcomatoid RCC as a distinct type of human RCC has changed over time. These tumours have been rarely described in cats and their biological behaviour a
nd immunohistochemical features are still uncertain.

Materials & Methods
This is a case report describing a unilateral tumour from a 7-year-old male domestic shorthair cat presented with a history of weight loss, azotaemia and unilateral renomegaly.

Results
Histologically, the kidney was diffusely effaced by streams, cords and aggregates of pleomorphic polygonal, spindle and binucleate/multinucleated cells. A preliminary diagnosis of SRCC, undifferentiated sarcoma, histiocytic sarcoma and undifferentiated carcinoma was made. The three c cell type weres strongly positive for vimentin and variably for AE1/AE3. Spindle and binucleate/multinucleated cells were Iba1-positive. Smooth muscle actin (SMA) immunostaining was strongly positive in spindle cells only. Immunostaining for CK7, HMWK, Melan A, p63, UPII and UPIII, were all negative, ruling out epitelioid angiomyolipoma and sarcomatoid urothelial cell carcinoma. Based on the literature reporting that (a) SRCC may have little or no pancytokeratin expression, with consistently strong vimentin expression, (b) cytoplasmic SMA immunoreactivity can be present in feline SRCC, and (c) the sarcomatoid component of human SRCC may assume histiocytic-like features, this case was classified as sarcomatoid RCC.

Conclusion
The mystery about feline sarcomatoid RCC is far from being solved! However, a basic panel of IHC markers (vimentin, AE1/AE3, Iba-1) is highly recommended to rule out any other possible differentials of mixed undifferentiated tumours of renal origin.

POSTER ABSTRACTS

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33 | AMYLOID OR NOT? CHARACTERIZATION OF DEPOSITS IN HYALINISING PANCREATIC ADENOCARCINOMA IN DOGS

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Background
Accumulation of amyloid and hyaline materials in various neoplasms among both animals and humans has been an intriguing area of study due to its diverse implications. Hyalinising pancreatic adenocarcinoma is histologically distinguished from conventional exocrine pancreatic carcinoma by intratumoral deposition of hyaline materials. Up to date, the origin of the hyaline material is unknown. This study analysed the proteomic nature of the hyaline material within pancreatic tumours in dogs.

Materials & Methods
Paraffin-embedded tissues from 8 dogs with pancreatic exocrine tumours underwent H&E, Congo red, and thioflavin S staining. The ultrastructure of the hyalinised lesions was analysed using TEM. Hyalinised deposits were collected from tissue sections, followed by LC-MS/MS analysis. Immunohistochemistry was conducted utilising an anti-lipase antibody.

Results
Histologically, 2 out of 8 dogs were diagnosed with hyalinising pancreatic adenocarcinoma. Hyalinised lesions were negative to Congo red. However, thioflavin S staining revealed moderate green fluorescence, whereas TEM detected non-branching amyloid-like fibrils. Mass spectrometry identified bile salt-activated lipase as the predominant component of these hyaline deposits. Immunohistochemistry results concurred with proteomic analysis, demonstrating that hyaline deposits exhibited positivity for lipase.

Conclusion
This study demonstrated that hyaline deposits in canine pancreatic adenocarcinomas have amyloid-like properties and their origin is bile salt-activated lipase. This study contributes to the understanding of this phenomenon and highlights the potential role of lipases in canine pancreatic tumours.

POSTER ABSTRACTS

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36 | RNASEQ TRANSCRIPTOME ANALYSIS IN RESPONSE TO DIFFERENT STRESSORS IN CANINE HISTIOCYTIC SARCOMA CELLS

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Background
To survive stress during development, self-renewal, or due to a changing environment including hypoxia, starvation or infections, cells utilize various pathways. Similarly, cancer cells also use these pathways to endure - stressor associated proliferation, to develop metastasis, and to avoid immune system reactions.

Materials & Methods
Canine histiocytic sarcoma cells (DH82 cells) were cultured under hypoxic and starving conditions as short (1 day) and long (3 days) term stressors. Subsequently, total RNA was extracted for RNA sequencing analysis (RNAseq) and obtained findings were compared to control conditions.

Results
In short-term cultures, 1645 differentially expressed genes (DEGs) were identified under hypoxic and 157 DEGs under starving conditions compared to controls. In long-term cultures, 1301 DEGs were found under hypoxic and 836 DEGs under starving conditions compared to controls. Moreover, cultures showed an increase in the number of DEGs over time, with 1323 DEGs in control cultures, 2609 DEGs under hypoxic, and 2149 DEGs under starving conditions. Interestingly, significantly altered genes mainly clustered in Gene Ontology (GO) terms associated with cell division, angiogenesis, inflammation response, metabolism, transcription and intracellular regulation.

Conclusion
In vitro induced stress leads to transcriptomic changes that increase with culture time. The GO terms are highly related to pathways associated with hallmarks of cancer including proliferation, metastasis, angiogenesis, and cell metabolism. Overall, the generated in vitro data provide important in-sights into tumor growth associated mechanisms and might provide important information for future therapeutic intervention concepts.

POSTER ABSTRACTS

Veterinary Pathology: Small Animal

46 | DESCRIPTION OF SPATIAL PROLIFERATION PATTERNS IN CANINE NEOPLASMS

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Background
In many canine malignancies, mitotic count (MC) is a prognostic factor. Standardization initiatives have defined the morphology of mitotic figures and size of the area, while selection of MC site within the tumor still needs standardization. This study aimed to evaluate the spatial arrangement of highest proliferative areas in canine tumors to define the best candidate areas for MC assessment.

Materials & Methods
Ki67 IHC was performed in canine melanomas, mast cell tumors (MCT), mammary carcinomas (CMC), and soft tissue sarcomas (STS). Using QuPath software Ki67 density maps were built on scanned slides, and classified as extension (focal/multifocal or diffuse) and as distribution (central, peripheral or scattered).

Results
Forty-three melanomas, 32 MCT, 23 CMC, and 87 STS were included. Ki67-distribution was peripheral in 89 cases, scattered in 82 and central in 14, correlating with diagnosis ($p<0.001$). In melanomas and CMC the distribution was mainly peripheral (77% and 52% respectively), in STS scattered (61%), while in MCT peripheral and scattered distributions were similar. Central distribution was evident in 26% of CMC, 16% of MCT, 3% of STS, and 0% of melanomas. Multifocality characterized 95% of the cases.

Conclusion
Based on these findings, highest proliferative areas should be searched at the periphery in melanomas while counting should not be limited to the periphery in STS and MCT. Finally, it should be considered that in 26% of CMC the highest proliferative area is central. These results represent a useful initiative to standardize the best tumor site for MC in canine tumors.

POSTER ABSTRACTS

Veterinary Pathology: Small Animal

48 | NON-INFILTRATIVE ANGIOLIPOMA IN A 15-YEAR-OLD FEMALE SPAYED CAT

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Background
Lipomas are common, predominantly subcutaneous benign tumours arising from well-differentiated adipocytes. Variants are uncommon and include for example fibrolipomas, angiolipomas and angiofibrolipomas, which can be infiltrative or not. Angiolipomas are rare and are composed of a mixture of adipocytes and endothelial cells. This case report describes a non-infiltrative angiolipoma in a cat.

Materials & Methods
A 15-year-old, female spayed, Persian x European Shorthair cat was presented with a solitary, subcutaneous mass on the back which was surgically excised. The tissue sample was fixed in 10% buffered formalin and routinely processed for histological examination.

Results
Macroscopically, the mass measured 3 centimeter in diameter, had a light brown colour and a moderately soft consistency on cut surface. Microscopically, elevating the dermis and epidermis and compressing the underlying musculature, a subcutaneous, well-circumscribed, non-encapsulated, expansile mass was effacing the normal tissue architecture. Approximately 80% of the tumor was composed of densely packed, mature, well-differentiated adipocytes, with clear cytoplasm and peripheral, flattened, hyperchromatic nuclei. Approximately 20% of the mass was composed of multifocal to coalescing clusters of branching blood vessels, lined by well-differentiated endothelial cells, partially obliterated by fibrin thrombi. The mass was completely excised with narrow margins.

Conclusion
Angiolipomas are rare benign tumors, with scarce data in veterinary literature and only few reports in dogs. In contrast with humans, where multiple angiolipoma nodules are common, dogs often have a solitary mass, as observed in this cat. This is the first report of a non-infiltrative angiolipoma in a cat, characterized by a good prognosis.

POSTER ABSTRACTS

Veterinary Pathology: Small Animal

54 | FATAL PULMONARY CRYPTOCOCCOSIS WITH DISSEMINATION TO THE ENCEPHALON IN AN IMMUNOCOMPETENT FELINE

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Background
Cryptococcus neoformans, C. gattii species complex pathogens associated with severe infections in humans and animals are acquired by inhalation of spores or dehydrated yeast cells from environmental sources. Whereas precise data concerning the prevalence of cryptococcosis in animals is not available, in humans more than 220,000 cases of this infection occur annually worldwide, resulting in up to 180,000 deaths. We report a case of sudden death in an indoor and immunocompetent female cat due to pulmonary cryptococcosis with dissemination to the encephalon.

Materials & Methods
A 2-year-old domestic short-hair female cat, negative for FeLV and FIV, was found dead without prior signs of illness. Tissue samples were collected for histopathology. Discriminatory immunohistochemistry (IHC) using monoclonal antibodies (471, 302, and F10F5) was performed in brain and lung sections for Cryptococcus species differentiation.

Results
Gross examination revealed a large deposition of body fat, moderate hypertrophy of retropharyngeal and mesenteric lymph nodes, generalised pulmonary congestion with consolidation areas and numerous nodules (1-2 mm) corresponding to round fungal structures with a PAS and Mucicarmin-positive capsule, with minimal inflammatory reaction. These fungal structures were also observed in the meninges with moderate infiltration of macrophages and in the neuroparenchyma inside cyst-like structures. IHC confirmed C. neoformans infection.

Conclusion
We report a rare C. neoformans pulmonary infection with meningoencephalitis in the absence of nasal disease in an immunocompetent cat. As the source of infection and strain-virulence factors could not be determined, investigation of environmental sources was recommended to minimize the risks of human and further animal infections.

POSTER ABSTRACTS

Veterinary Pathology: Small Animal

55 | FELINE GIANT CELL GLIOBLASTOMA MIMICKING A MENINGEAL PLEOMORPHIC SARCOMA

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Background
Gliomas are the second most common feline primary intracranial neoplasms. Giant cell glioblastoma (GC-GBM) is an infrequent subtype of GBM accounting for less than 1% of human gliomas. Only 3 cases have been diagnosed postmortem and published in 2 dogs and 1 cat. We describe the first surgically excised and antemortem diagnosed feline GC-GBM.

Materials & Methods
A 13-year-old domestic shorthair cat was presented with acute onset neurological signs of forebrain dysfunction. Magnetic resonance imaging revealed a well circumscribed, 2.0x1.2x1.1 cm, extra-axial lobulated mass attached to the falx cerebri, compressing thalamus and parietal lobes. Complete surgical resection was grossly achieved. Samples were routinely processed and stained with HE. Immunohistochemical stains were performed against GFAP, S100, pancytokeratin, vimentin, SMA, desmin, Iba1, Olig-2, neurofilaments, synaptophysin and Kli67.

Results
Histopathology revealed a highly cellular, infiltrative, neoplastic proliferation of atypical, pleomorphic cells including giant multinucleated and karyomegalic cells with bizarre nuclei, in a disorganized pattern with multifocal streaming bundles, and multifocal necrosis. Mitotic count and Ki67 labelling were high, with numerous atypical mitoses. Most cells were positive for vimentin, GFAP, Olig-2 and E-cadherin, while few stained for S100 and rarely for neurofilaments. Surgical recovery with progressive improvement of neurological signs was observed but the animal relapsed 2,5 months later, and was euthanized.

Conclusion
GC-GBM should be considered as a differential diagnosis for highly anaplastic intracranial neoplasia, as clinicopathological features might overlap with meningeal sarcomas or anaplastic meningiomas. High E-cadherin expression is extremely rare in human gliomas and has never been reported in canine or feline gliomas.

POSTER ABSTRACTS

Veterinary Pathology: Small Animal

79 | ORAL INFLAMMATORY MYOFIBROBLASTIC TUMOUR IN A DOG

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Background
Inflammatory myofibroblastic tumour (IMT) is a rare entity described in the subcutis, orbital soft tissue and the nasal cavity of dogs. Lesions are discrete masses composed of a mixture of bland fusiform myofibroblastic cells and inflammatory infiltrates composed of varying proportions of lymphocytes, plasma cells and macrophages. An oral palate/tonsillar sample of a 12-years-old, male, Catalan Sheepdog was submitted for histopathologic examination.

Materials & Methods
The sample was fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin and eosin. Immunohistochemical detection of vimentin, calponin, alpha-smooth muscle actin and desmin was accomplished using a standard protocol.

Results
Histopathology revealed a well demarcated mass consisting of interweaving and interlacing streams and bundles of spindle cells admixed with numerous mature lymphocytes, scattered plasma cells and macrophages. Mitoses were rare. All the spindle cells reacted strongly to vimentin. The cells also showed marked cytoplasmic positivity for desmin. The spindle cells were negative for both calponin and alpha-smooth muscle actin. Immunohistochemistry results were consistent with a myoid derivation for the spindle-cell population and the diagnosis of inflammatory myofibroblastic tumour was made.

Conclusion
To the authors knowledge, this is the first report of oral inflammatory myofibroblastic tumour in veterinary literature. Masses corresponding to human IMTs exist in animals and will be diagnosed with increasing frequency as more unclassified spindle-cell masses are examined immunohistochemically. This diagnosis should be considered for any spindle-cell mass with a prominent inflammatory infiltrate and the particular immunohistochemical panel described above.

POSTER ABSTRACTS

Veterinary Pathology: Small Animal

81 | PATHOLOGY AND IMMUNOHISTOCHEMISTRY OF SARCOMATOID RENAL CARCINOMAS IN DOGS

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Background
Sarcomatoid renal cell carcinoma (SRCC) is a subtype of primary renal tumor characterized by the proliferation of both epithelial and mesenchymal-appearing components, as well as the loss of the epithelial features of renal cell carcinoma. The phenomenon of epithelial-mesenchymal transition (EMT) has been proposed as a histogenetic mechanism in humans. We investigate the morphologic and immunohistochemical features of SRCC in dogs, and the possible role of EMT in its development.

Materials & Methods
Nine cases of canine SRCC were evaluated grossly and microscopically to define key morphological criteria. Immunohistochemistry was performed against cytokeratins, vimentin, CD-10, protein 14-3-3s and E -Cadherin. Immunohistochemical expression was evaluated semiquantitatively.

Results
SRCCs appeared as irregular or heterogeneous masses of solid and firm consistency, with frequent areas of mineralization. Most of them manifested a tubular histological growth pattern with osteosarcomatous type differentiation. Average mitotic count was 4 times higher in the mesenchymal compared to the epithelial component. Cytokeratins and vimentin were expressed in all cases (9/9; 100%), 8/9 cases (88.8%) were positive for E- Cadherin and 14-3-3s protein, and CD10 showed a reaction in 7/9 cases (77.8%). As the neoplastic cells underwent transformation from a carcinomatous to a sarcomatoid phenotype, they showed increased expression of vimentin and a marked or total loss of E-Cadherin expression.

Conclusion
These preliminary results confirm the renal epithelial origin of SRCCs and transformation of the carcinomatous component into sarcomatoid, suggesting an aggressive phenotype and involvement of EMT mechanism in their histogenesis.

POSTER ABSTRACTS

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82 | CLINICOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL CHARACTERISTICS OF MESOTHELIOMAS OF THE TUNICA VAGINALIS TESTIS IN DOGS (2000-2023)

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Background
The tunica vaginalis testis is an extension of the peritoneum normally lined by a single layer of mesothelial cells. Mesotheliomas of the tunica vaginalis are extremely rare. We aim to define the clinical, histological and immunohistochemical characteristics as well as the prognosis of this neoplasia in dogs.

Materials & Methods
A systematic review of our records looking for animals with a diagnosis of testicular mesothelioma made between 2000 and 2017 revealed 13 cases. Clinical data and follow-up were obtained through email and telephone interviews with referring clinicians. Diagnoses were confirmed by review of HE-stained, paraffin-embedded specimens, and immunohistochemically with a panel of antibodies including cytokeratins, vimentin, and CEA.

Results
All cases presented as scrotal swelling. Scrotal ultrasound evidenced irregular peritesticular masses with heterogeneous echogenicity, mostly accompanied by hydrocele. Postoperative recovery was favourable; however, the existence of abdominal effusion and worsening of the general condition before 7 months due to metastatic dissemination was common. Gross examination revealed thickening of the tunica vaginalis studded by variable size, multiple, firm, nodular or polypoid lesions with a solid cut surface. Invasion of the spermatic cord was common. Occasionally, the tunic showed diffuse calcification. Microscopically, the epithelioid subtype with papillary growth pattern stood out among others and all tumours consistently expressed pancytokeratin, cytokeratins 5/8 and vimentin.

Conclusion
Mesothelioma of the testicular tunica vaginalis in dogs is a rare, highly malignant neoplasm, with aggressive behaviour and a poor prognosis.

POSTER ABSTRACTS

Veterinary Pathology: Small Animal

84 | A RETROSPECTIVE ANALYSIS OF 351 TESTICULAR TUMOUR DIAGNOSES IN AUSTRALIAN PET DOGS (1992-2022)

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Background
The epidemiology of testicular tumours has not been investigated in the Australian canine population. This retrospective cross-sectional study aims to characterise its occurrence in terms of demographic features, and spatiotemporal distribution between 1992 and 2022.

Materials & Methods
Data were collected from VetCompass Australia, a nationwide electronic patient database collating veterinary consultation records on companion animals attending Australian general practices. Keywords were used to identify patient histories with data on testicular tumours which were then manually screened for histopathological diagnosis. The association between types of testicular tumours and potential risk factors was analysed with Pearson's chi-squared test in R and ArcGIS was used to visualise the location of clinics where cases were recorded.

Results
A total of 351 testicular tumours were identified involving 333 canine patients. Tumour types included 115 seminomas, 113 sustentacular (Sertoli) cell tumours, 76 interstitial cell tumours, 40 mixed germ cell-sex cord stromal tumours, and seven mixed sex cord stromal tumours. The three most diagnosed breeds were the Golden Retriever (8.8%), Staffordshire Bull Terrier (6.0%), and German Shepherd (4.8%). Mean age at time of diagnosis was 10.2 years old. Cryptorchidism was present in 20.2% of cases and a statistically significant difference between presence of cryptorchidism and tumour type was found ($P<0.001$). Clinics that recorded testicular cancer diagnoses were located mainly in urban and suburban areas around major cities.

Conclusion
This study provides the most comprehensive data on the epidemiology of testicular tumours in Australian pet dogs.

POSTER ABSTRACTS

Veterinary Pathology: Small Animal

87 | CONCOMITANT INTESTINAL LYMPHOMA AND SQUAMOUS CELL CARCINOMA IN A CAT: A CASE REPORT

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Background
Alimentary lymphoma is the most common neoplasm of the digestive tract in cats. Enteropathy-associated T-cell lymphoma type 2 (EATCL II) which is a low-grade form characterized by T small cell lymphocytes is the most frequently subtype. Intestinal adenocarcinoma is the second most common neoplasm in this species, but squamous differentiation or primary squamous cell carcinoma is not described in veterinary literature and rare in human medicine.

Materials & Methods
A 14-year-old short-haired cat had a history of gastrointestinal alterations. At ultrasound examination, a diffuse enlargement with a focal area of consolidation was affecting the small intestine. Full thickness biopsy samples were taken from stomach, duodenum, ileum, and the mesenteric lymph node, including a large segment of ileum which showed a protruding mass. Immunohistochemistry was performed including CD3, CD20, Cytokeratin 5/6 (CK5/6), p16 and p63.

Results
Histological, a diffuse neoplasm consisteding of small lymphocytes affected transmurally all intestinal segments. The lymph node was also affected. There was a second infiltrative mass focally affecting the ileum which consisted of an epithelial cell population arranged in cords and nests with squamous differentiation. Neoplastic lymphocytes were CD3 positive whereas the epithelial neoplastic population was strongly positive to CK5/6 and p63.

Conclusion
Tumour collision is an occasionally described event in veterinary literature. The histochemical and immunohistochemical studies in this case showed a transmural small T cell lymphoma in concomitance with an epithelial neoplasm consisting of a primary squamous cell carcinoma of the intestine, which has not been previously described in veterinary literature.

POSTER ABSTRACTS

Veterinary Pathology: Small Animal

88 | COMBINED HEPATOCELLULAR CARCINOMA AND CHOLANGIOCARCINOMA, WITH SARCOMATOID TRANSFORMATION: HISTOLOGICAL AND IMMUNOHISTOCHEMICAL DESCRIPTION

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Background
Cases of a double cancer type and a mixed type are occasionally described in veterinary literature. Hepatocellular carcinoma in coexistence with cholangiocarcinoma is an extremely rare phenomenon described in domestic species, being also rare in human medicine. In epithelial-mesenchymal transition, epithelial cells undergo multiple changes to acquire a mesenchymal-like phenotype characterized by enhanced migratory capacity, invasiveness, and increased production of extracellular matrix components.

Materials & Methods
A 4-year-old Shih tzu was euthanized due to hepatic alterations and the presence of a neoplasm in the liver. A complete histological and immunohistochemical study was performed including cytokeratins 7 (CK7) and AE1/AE3, E-cadherin, CD10, vimentin, CD31, smooth muscle actin, HepPar1, CD56, Ki67 and CD44.

Results
Microscopically, the neoplasm was composed of three neoplastic populations consisting of a hepatocellular carcinoma, a cholangiocarcinoma and a third mesenchymal population with sarcomatoid features and areas of osteoid matrix. Hepatocellular portions were diffusely HepPar1 positive and cholangiocellular areas were highly positive for CKs CK7 and AE1/AE3 and moderately to vimentin and CD56 in more atypical portions. Both neoplastic components were E-cadherin positive. The mesenchymal areas showed strong positivity for vimentin, CD56, CD10 and mild (in transition areas) to scant (in sarcomatoid) positivity to E-cadherin. All three populations were strongly positive for Ki67.

Conclusion
Immunohistochemical studies were compatible with loss of expression of epithelial-related markers and acquisition of mesenchymal-related markers as sarcomatoid phenotype developed showing evidence of epithelial to mesenchymal transition. The sarcomatoid portions appeared to be arisen from cholangiocellular neoplastic component.

POSTER ABSTRACTS

Veterinary Pathology: Small Animal

95 | PULMONARY MASS IN A DOG - DOES NEGATIVE ZIEHL-NEELSEN STAINING RULE OUT MYCOBACTERIOSIS?

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Background
Mycobacteriosis is a potential zoonotic disease routinely diagnosed in histology by Ziehl Neelsen (ZN) staining. Long standing antibiotic treatment can cause failure of the ZN staining to reveal the mycobacteria due to a disrupted bacterial capsule. This poster describes a canine case of pulmonary mycobacteriosis with loss of acid-fast bacterial properties.

Materials & Methods
A 3-year-old intact border collie bitch with severe lobar pneumonia, non-responsive to antibiotic treatment of several weeks duration, underwent surgical lobectomy of the middle right lung lobe. The lobe was sent for pathological examination to identify the cause of the pneumonia. HE, ZN, modified ZN; Grocott's methenamine silver stain (GMS) and in-situ hybridization (ISH) with probe detecting bacterial 16S rRNA were performed. Microbiological culture was performed from the lung biopsy, bronchoalveolar lavage (BAL) fluid and thoracic cavity effusion.

Results
HE staining revealed severe confluent chronic pyogranulomatous pneumonia in the resected lung lobe. ZN and modified ZN staining were negative in all sections. GMS revealed bacillus-like structures within the granulomas and chromogenic ISH confirmed these to be bacteria. Bacterial growth was seen in the BAL fluid after six days incubation and in the biopsy and pleural effusion after two weeks incubation. The cultured bacterium was identified as Mycobacterium thermoresistibile by mass spectrometry.

Conclusion
This case highlights the importance of molecular methods for detecting bacterial infection, in addition to standard histochemical stains, in cases with a history of prolonged antibiotic treatment and a persistent tissue lesion suggestive of infection.

POSTER ABSTRACTS

Veterinary Pathology: Small Animal

99 | VALIDATION OF A DIAGNOSTIC ALGORITHM FOR THE DIAGNOSIS OF CANINE AND FELINE MAMMARY TUMORS: UPDATE ON AN ONGOING STUDY

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Background

Focusing on the standardization of the diagnoses of mammary neoplastic and hyperplastic lesions in dogs and cats, a graphical diagnostic algorithm based on the Davis-Thompson Foundation (DTF) classification has been proposed by a panel of 17 veterinary pathologists, either authors of the DTF classification and/or of the Italian guidelines on mammary tumors. To assess its effectiveness in standardizing diagnoses and improving inter-observer agreement, an international ring test study was prepared.

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Materials & Methods
Digitally scanned histological slides of 112 canine and feline neoplastic and hyperplastic lesions of the mammary gland were selected. Participants were recruited through congresses, webinars and personal contacts. They were requested to read and grade each slide twice, initially using the DTF classification and then strictly following the algorithm. Results will be statistically analysed to assess concordance among readers and agreement with the gold standard diagnoses derived from the consensus diagnoses of the panel.

Results
Ninety-seven pathologists from 23 different countries and 4 continents agreed to participate as readers. Thirty-two are junior (with less than 3 years of diagnostic experience, without a diploma in veterinary pathology) and 65 are senior pathologists (39 holding a diploma in veterinary pathology).

Conclusion
The broad international participation of both young and senior pathologists from different continents is remarkable and underscore the significant interest in standardizing the diagnostic approach and in participating in collaborative studies. This might represent a first extensive international concordance study for mammary tumors in dog and cat, potentially fuelling an international discussion on critical issues.

POSTER ABSTRACTS

Veterinary Pathology: Small Animal

113 | MALIGNANCY ANALYSIS IN 26,224 HISTOPATHOLOGIC DIAGNOSES OF CANINE TUMORS: RESULTS FROM A MULTICENTREER STUDY IN CENTRAL ITALY (2008-2023)

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Background

Animal Cancer Registries (ACRs) significantly enhance our understanding of oncology, providing insights into epidemiologic and clinical trends, and fostering comparative research. This study aimed to provide data on a large set of canine tumours, analysing their distribution and malignancy profile by risk factors.

Materials & Methods

Vet-ICD-O-canine-1 was used to code 26,224 histologically diagnosed tumours from two pathology-based ACRs in central Italy between 2008 and 2023. The Cochrane-Armitage test and logistic regression analysis were performed to investigate the influence of different variables on the binary outcome (malignant versus benign tumours). Odds ratios (ORs) for specific histotypes were reported.

Results

Benign and malignant tumours were 10,902 (41.6%) and 15,316 (58.4%) out of the total, respectively. The risk of malignancy was shown to increase by 8% with each year of age. Females had a higher risk of developing a malignant tumour (OR = 2.16, CI95% 2.04-2.29). Neutering status didn't affect overall malignancy but contributed to histotype-specific risks. The ORs of developing malignant tumours and specific histotypes were largely influenced by breed. The Dogo Argentino had the highest malignancy risk (OR = 2.18, CI95% 1.49-3.25), with an increased risk for squamous cell tumours (OR = 5.15; CI95% 3.35-7.71), while the West Highland White Terrier had the lowest (OR = 0.54, CI95% 0.38-0.76), and an increased risk for histiocytic tumours (OR = 3.03, CI95% 1.35-6.08).

Conclusion

This study confirmed previous findings, revealed novel potential risk factors and breed predispositions, and highlights the value of multicentre collaboration in identifying high-risk oncology patients.

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Veterinary Pathology: Small Animal

114 | PRELIMINARY STUDY ON TUMOR-ASSOCIATED MACROPHAGES IN FELINE ALIMENTARY LYMPHOMAS

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Background
Interactions between tumor cells and tumor-associated macrophages (TAMs) appear to have significant effects on treatment response and prognosis in oncological patients, as testified by previous studies both in human and veterinary medicine. This study aimed to characterize TAMs in feline alimentary lymphomas.

Materials & Methods
Thirty-four feline alimentary lymphomas were retrieved from the Animal Cancer Registry of the Department of Veterinary Sciences, University of Pisa. Histopathology was carried out to classify lymphomas as mucosal or transmural. Immunohistochemistry was performed to ascertain the immunophenotype (anti-CD3 and CD20 antibodies), and to highlight total (anti-Iba-1), M2-polarized (anti-CD204), and recently recruited (anti-MAC387) macrophages. Immunostained cells were counted in 2.37 mm^2 and M1-polarized calculated as the difference between total and CD204-positive macrophages.Mann-Whitney U test, Kruskall-Wallis test and Dunn's post-hoc analysis were used to assess differences among tumor groups.

Results
Thirty-three lymphomas were classified as 22 transmural lymphomas (7 B-cell, 12 T-cell, 3 non-B non-t neoplasms), and the remaining 11 as T-cell mural lymphomas. Iba1-, CD204-, and MAC387-positive macrophages were higher in transmural lymphomas compared to mucosal ones ($p<0.01$, $p<0.01$, and $p<0.05$, respectively). CD204-positive cells were higher in T-cell lymphomas ($p<0.01$), while differences in M1-polarized macrophages resulted not significant.

Conclusion
Our results are comparable to previous studies on canine lymphoma and highlight the need to further clarify the role of TAMs and the tumor microenvironment in feline alimentary lymphomas.

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124 | INTRA-TUMORAL HETEROGENEITY OF ANISOKARYOSIS IN CANINE CUTANEOUS MAST CELL TUMOURS

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Background
Anisokaryosis of tumour cells is an important histological malignancy criterion. For canine cutaneous mast cell tumours (ccMCT) anisokaryosis, referred to as “karyomegaly”, is routinely evaluated as part of the grading system. This study aimed to evaluate the distribution of anisokaryosis throughout the tumour section (intra-tumoral heterogeneity) to investigate whether 1) region selection influences anisokaryosis evaluation and 2) heterogeneity of anisokaryosis is prognostically relevant.

Materials & Methods
Anisokaryosis was evaluated in whole slide images (WSI) of 96 ccMCT with known tumour-specific death (TSD). A previously established deep learning-based algorithm was used to measure the area of all tumour nuclei within the manually or algorithmically segmented tumour area of each WSI. The standard deviation (SD) of nuclear size was calculated for small tumour regions each containing 112 cells on average.

Results
For a single, manually selected tumour region, the SD of nuclear area had an area under the curve (AUC) of 0.943 for TSD. Intra-tumoral distribution analysis of SD of nuclear area revealed higher values and a larger spread of values for cases with TSD. Cases with TSD had on average 44.4% hotspots (locations above the threshold of ≥11.5 µm²), while the other cases had on average 5.8% hotspots. The percentage of hotspots was indicative for TSD (AUC = 0.948).

Conclusion
This study demonstrated variable intra-tumoral distribution of anisokaryosis in some cases indicating that region selection can influence prognostication. Further studies should therefore evaluate the influence on inter-rater reproducibility. Intra-tumoral heterogeneity of anisokaryosis seems to carry good prognostic information.

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129 | EXPLORATION OF DISEASE-RELATED SEQUENCES IN AA AMYLOIDOSIS IN MIXED-BREED CATS

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Background
AA amyloidosis is characterised by functional impairment due to abnormal deposition of misfolded serum amyloid A (SAA) protein in various organs. While some pure-breed cats are known to inherit AA amyloidosis, there has been a growing recognition of its occurrence in mixed-breeds. This analysis aims to identify disease-associated genes by comparing SAA genes in cats with and without AA amyloidosis.

Materials & Methods
SAA sequences from five AA amyloidosis-affected and five amyloidosis-free mixed-breed cats were sequenced. 3D structural differences in each sequence were predicted using AlphaFold2 software. Hepatic and renal amyloid deposits were subjected to LC-MS/MS to identify SAA sequences that form amyloid in tissues.

Results
Irrespective of amyloidosis, seven polymorphisms in mature SAA sequences were found in mature SAA sequences at positions 1(Gln/Glu), 29(Lys/Ile), 42(Asp/Glu), 45(Gln/Arg), 48(Pro/Arg), 51(Ala/Val), and 75(Asn/Ser) in ten cats. Each SAA polymorphic sequence did not show major differences in the predicted 3D structure. Based on LC-MS/MS, Ile29, Asp42, Pro48, and Ala51 were detected from amyloid, but peptides including Lys29, Glu42, Arg48, and Val51 were not detected. For the other three polymorphic sequences, amyloid-specific sequences were not detected in the amyloid components.

Conclusion
The feline SAA sequences were characterised by various polymorphisms, with no allele-specific for cats with amyloidosis and no differences in the predicted 3D structure. However, it was revealed that the sequences comprising AA fibrils may be specific SAA sequences derived from a combination of polymorphic sequences.

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132 | PULMONARY ADENOCARCINOMA WITH THORACIC WALL IMPLANTATION IN A CAT (FELIS CATUS)

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Background
Lung adenocarcinomas are rare neoplasms in cats with a poor prognosis due to metastasis to mediastinum, lymph nodes and digits. Highly undifferentiated neoplasms and proven metastasis are known factors that reduce the survival time. In humans and dogs pulmonary adenocarcinoma implantation has been reported after fine-needle aspiration cytology (FNA) or surgery.

Materials & Methods
A twelve-year-old, female, domestic cat with anorexia and vomiting was presented with a fast-growing mass in the subcutaneous tissue associated with the thoracic wall. Three months prior, after cytological and histological diagnosis of adenocarcinoma, a partial lung lobectomy was performed. Due to prognosis and other comorbidities (renal disease), euthanasia was elected, and a full postmortem study was performed. The samples were subsequently processed for histopathology and immunohistochemistry (for markers TTF-1 and pancytokeratin).

Results
The thoracic wall tumor was consistent with a metastatic adenocarcinoma. Additional metastases were found in the pleura, other lung lobes and mediastinal lymph nodes. Histologically, cellular atypia resembled that of the adenocarcinoma from the lobectomy. There was also severe desmoplastic response. Neoplastic epithelial cells were positive for TTF-1 and pancytokeratin in the lung, confirming a pulmonary origin. Other relevant findings were a 130 mL hydrothorax, glomerulonephritis, and interstitial nephritis.

Conclusion
The ribs are rarely reported as metastases sites in feline pulmonary adenocarcinomas. The latter together with the development of the neoplasm close to the site of resection raises the possibility of the tumor being seeded during the FNA or the surgical procedure.

POSTER ABSTRACTS

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133 | EPIDEMIOLOGICAL DESCRIPTION OF MULTIPLE MALIGNANT PRIMARY TUMORS IN A SUBSET OF THE ITALIAN DOGS POPULATION

C. Cocumelli[1], N. Fonti[2], V. Galietta[1], A. Carnio[1], A. Carvelli[1], C. Eleni[1], F. Ingravalle[3], E. Manuali[4], C. Maresca[4], F. Millanta[2], P. Scaramozzino[1], S. Simeoni[1], M. Vascellari[5], C. Zanardello[5], M. Ines Crescio[3]

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Background

Multiple malignant primary tumours (mMPTs) occur in humans with frequencies ranging from 2 to 17%; the scarce and dated publications in pets report similar frequencies. This collaborative study aims at quantifying the frequency and association between mMPTs in dogs in Italy.

Materials & Methods

Cases were collected from four Italian Animal Cancer Registries (Lazio, Umbria, Vicenza and Venezia provinces, Toscana) from ten years. Criteria for selection were dogs with at least two distinct primary tumours, at least one of them malignant, regardless the time of diagnosis. Associations between different types of tumours in cases were assessed by means of chi-square; Poisson regression to assess influence of sex, age, race and sexual status were performed.

Results

Between 2013-2022, 20,213 tumours in 18,916 dogs were recorded; 803 dogs had mMPTs (4.25%), 63% were females and 66% were intact (55% of which were females). The risk to develop mMPTs increases with age and in intact dogs. For topography, mammary gland in females and skin in both sexes are overrepresented; strongly associated tumours ($p < 0,03$) are specialized gonadal tumours with mast cell, adnexal, soft tissue, and blood vessels tumours in males while in females, mammary tumors are associated with mast cell, soft tissue, and squamous cell tumours ($p < 0,03$).

Conclusion

These preliminary results highlight strong associations between morphologic categories of tumours, with differences between males and females. Studying MPTs can highlight differences in risk between oncological and general population, increase knowledge or speculate on new pathogenetic hypotheses on tumours that share the same exposures.

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142 | URINARY BLADDER MAST CELL TUMOUR WITH NON-AGGRESSIVE CLINICAL COURSE IN A DOG: AN UNEXPECTED CASE

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Background
Although visceral canine mast cell tumours (cMCTs) are generally associated with a worse prognosis, the only reported case of cMCT affecting the urinary tract (ureter) of a dog did not show clinically aggressive behaviour.
The aim of our study was to describe a cMCT affecting the urinary bladder.

Materials & Methods
During routine periodical abdominal ultrasonography on an 11 year old neutered male dog, urinary bladder examination revealed a small nodule projecting into the lumen. A full-thickness urinary bladder biopsy was then referred to a private veterinary diagnostic laboratory in December 2023. After routine tissue processing, FFPE samples were stained with HE and Giemsa, and immunohistochemistry for KIT protein was performed.

Results
Histopathology revealed an exophytic nodule lifting the upper lamina propria, composed by sheets of monomorphic small round cells with minimal atypia and no mitoses, admixed with fewer eosinophils. Giemsa stain and KIT immunohistochemistry revealed cytoplasmic intense granularity and membranous (KIT pattern I) positivity, respectively, confirming cMCT diagnosis. The patient suffered only of mild age-related lameness; blood test was unremarkable and no cutaneous lesions were present. Although a complete staging was not performed, two postoperative ultrasound checks were unremarkable, and the dog was clinically healthy to the last follow-up (April 2024).

Conclusion
To the best of our knowledge, this is the first report of a urinary bladder cMCT. Despite its visceral localization and similarly to the only previously reported cMCT affecting the ureter, the neoplasm here described did not show an aggressive behaviour until the last follow-up.

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144 | CANINE MAST CELL TUMORS: PREDICTION OF THE C-KIT EXON 11 GENOTYPE BY PHENOTYPE - HUMAN OBSERVER VS ARTIFICIAL INTELLIGENCE

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Background
Mast cell tumors (MCTs) are frequent neoplasia of dogs with variable biological behavior. Internal tandem duplication mutations in c-Kit exon 11 (c-Kit-11-ITD) are associated with poor prognosis but predict therapeutic response to tyrosine kinase inhibitors. In a previous work, a deep learning algorithm managed to predict the presence of c-Kit-11-ITD on digitalized HE-stained histological slides (Whole Slide Images, WSIs) in up to 87% of the cases, suggesting the existence of morphological features characterizing MCTs carrying this mutation.

Materials & Methods
In an ongoing three-stage blinded study, three untrained pathologists were first asked to classify eight WSIs and 200 patches of MCTs in c-Kit-11-ITD positive or negative. Second, they were trained to recognize c-Kit-11-ITD by having access to a set of WSIs with known mutational status and 200 patches of areas highly relevant for algorithmic c-Kit-11-ITD classification. Third, pathologists were asked to classify 16 new WSIs and 200 new patches for c-Kit-11-ITD status. Participants had to report the microscopic features they identified as relevant for their decision.

Results
The participants correctly classified the c-Kit-11-ITD status of 63–88% of the WSIs and 43-55% of the patches without training, but only 25-38% of WSIs and 55-56% of patches after training. Nuclear pleomorphism was commonly named as potential feature of c-Kit-11-ITD positive MCTs.

Conclusion
Based on the current results it is assumed that transfer of algorithmic skills to the human observer is difficult. A c-Kit-11-ITD specific morphological feature usable for human observers remains thus to be extracted from the AI-model.

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147 | BILIARY HAMARTOMA IN A DOG: HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL ANALYSIS

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Background
Biliary hamartoma (Von Meyenburg complex) represents a congenital disorder of the intrahepatic biliary system, along with congenital hepatic fibrosis and Caroli's disease. These ductal plate malformations are rarely encountered in animals, with only a few cases described in the literature. This report aims to describe the histological and immunohistochemical features of this uncommon tumour like mass.

Materials & Methods
An 18-year old male mixed breed dog was presented for post-mortem evaluation. The necropsy exam revealed an incidental solitary cystic hepatic mass, measuring approximately 12 cm in diameter. Samples were collected for microscopical evaluation, using hematoxylin-eosin and Masson's trichrome stains for histological examination, followed by immunohistochemical assessment, using antibodies for multi-cytokeratin, cytokeratin 19, carcinoembryonic antigen and Ki-67.

Results
Histologically, the hepatic parenchyma was distended and compressed by a non-encapsulated, well-demarcated, multi-cystic mass, consisting of many medium to large-sized cystic spaces and a network of anastomosing trabecular projections, interpreted as proliferating biliary ducts. The cysts were lined by a single layer of cuboidal cells, showing minimal cellular pleomorphism. Multifocal, groups of hepatocytes were observed entrapped by the proliferating cystic ducts. The proliferating cells showed strong immunopositivity for multi-cytokeratin, cytokeratin 19, and carcinoembryonic antigen, and a low Ki-67 index (1%). Based on the microscopical aspect, the differential diagnoses included biliary cystic adenoma and Caroli-like disease. The results of the immunohistochemical exam, corroborated with the histological aspect, supported the diagnosis of biliary hamartoma.

Conclusion
While typically small and seldom clinically significant, biliary hamartomas should be considered as a differential diagnosis, when assessing large cystic lesions of the liver.

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170 | BRAIN INVOLVEMENT IN HIGHLY METASTATIC CANINE ORAL FIBROSARCOMA

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Background
Oral neoplasia represents up to 5% of all dog neoplasms, with fibrosarcomas being the third most common malignant oral neoplasms. Oral fibrosarcomas tend to be locally aggressive and invasive despite their histological characteristics, but they are slow to metastasize.

Materials & Methods
A 9 year-old cross breed female dog presented to the hospital with a foreign body in the left mandibular branch. After CT-scan a mass was observed affecting the jaw and regional lymph nodes. Affected tissues were excised and submitted for histopathological examination. The dog returned to the hospital after 13 months with masses in the heart, right medial lung lobe and thalamus and was humanely euthanized. A complete necropsy was performed, and samples of the main organs were collected for histopathological evaluation.

Results
The mandibular biopsy sample contained an osteolytic, infiltrative poorly delimited mesenchymal neoplasia. Neoplastic cells grew forming dense multiple-direction streams and had little eosinophilic cytoplasm. There were 17 mitosis in 2.37mm2. Anisokaryosis and anisocytosis were moderate. The masses in the lung, brain, and heart were confirmed to have the same histological characteristics as the oral mass. Neoplastic cells were intensely positive to vimentin but negative to desmin, pancytokeratin, and melanA immunostainings, leading to a diagnosis of metastasized oral fibrosarcoma.

Conclusion
The presentation of this canine oral fibrosarcoma was atypical. First, maxillary bone is more commonly affected than mandible. More importantly, despite high local invasion and recurrence, metastases are rare, while in this case there was multiorgan spread involving the brain, lung, and heart.

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173 | A CASE OF FELINE INTRAOCULAR NEOPLASM WITH NEURAL CREST DERIVATION

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Background
Detailed characterization of intraocular and periocular neoplasms in cats can be a nuisance due to paucity of reports and complexity of this anatomical site. Here we report a still unidentified feline intraocular neoplasm with a slowly-progressing and locally-aggressive clinical behaviour.

Materials & Methods
In March 2022 a 10 year old neutered male cat with history of previous intraocular antibiotic injections, was referred to a private veterinary clinic for an intraocular mass. The enucleated eye-globe was submitted for histopathology to a private diagnostic laboratory. Routinely processed FFPE samples were stained with HE and Alcian-blue, and different immunohistochemical markers were investigated.

Results
Histopathology revealed a completely excised neoplasm expanding into the vitreous chamber and totally effacing retinal architecture. The neoplasm was composed of long palisading spindle cells with mild atypia, arranged in lobules embedded within mucinoid matrix with multifocal chondroid metaplasia. At immunohistochemistry, neoplastic cells were positive for vimentin, S100, GFAP, and NSE, and negative for pancytokeratin, synaptophysin, and melanocytic markers. After two years (March 2024) a histologically overlapping neoplasm recurred within the orbit and extending into oral cavity, leading to euthanasia of the cat.

Conclusion
Immunohistochemical results indicate a neural crest derivation for the neoplastic cells. According also to its anatomical distribution, the neoplasm might have originated from retinal cells (e.g. Müller cells) thus supporting a diagnosis of intraocular glioma, as rarely reported in dogs. On the other hand, the potential post-traumatic aetiology of the neoplasm implies a feline post-traumatic sarcoma with neural crest derivation as the main differential diagnosis.

POSTER ABSTRACTS

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175 | PROGNOSTIC VALUE OF PDL1 EXPRESSION AND T LYMPHOCYTE INFILTRATES IN CANINE MAMMARY CARCINOMAS

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Background
Programmed death cell protein 1 (PD1) is an immune checkpoint molecule expressed on the surface of T cells. When it binds to programmed death cell ligand 1 (PDL1), negative signals are propagated to T cells, initiating an essential mechanism of suppression for controlling the immune response. This mechanism can also be used by tumour cells, which can express the PDL1 protein and use it as a mechanism to evade the immune response. However, there is scarce information about the prognostic value of PDL1 in canine mammary carcinomas (CMC). The objective of this study was to characterize the expression of PDL1 and the cytotoxic (CTL) and regulatory T lymphocyte (RTL) infiltrates in CMC in order to verify their prognostic value.

Materials & Methods
Seventy-seven CMC samples were analysed histologically and the expressions of PDL1, CD8 and FOXP3 were detected using immunohistochemistry. The percentages of positive intratumoral CTLs and RTLs were obtained from five high-power field images (0.08 mm^2 per image) from hotspot areas. For PDL1, tumours with ›10% of labelled cells were considered positive.

Results
PDL1-positive carcinomas showed higher CTL intratumoral infiltrates ($p = 0.0482$). Bitches with PDL1-negative tumours had shorter post-surgical survival ($p = 0.0320$). Post-surgical survival was also shorter for bitches with highly RTL-infiltrated carcinomas ($p = 0.0081$).

Conclusion
Our findings indicate that PDL1 positivity might serve as an indicator of a more favourable prognosis in dogs with mammary carcinomas, while the presence of intratumoral RTL infiltrates could be a negative prognostic factor.

POSTER ABSTRACTS

Veterinary Pathology: Small Animal

181 | CD30 EXPRESSION IN CANINE CUTANEOUS MAST CELL TUMOURS

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Background
Mast cell tumour (MCT) is a frequent malignant skin neoplasm in dogs. Histopathological grading is the primary prognostic tool for this tumour. CD30 is an immune cell marker of the tumour necrosis factor receptor family. It is considered a new therapeutic target in human mast cell neoplasms and a potential marker for canine MCTs. The purpose of this study was to characterise the expression of CD30 in canine cutaneous MCTs and to evaluate its potential as a prognostic marker for the disease.

Materials & Methods
Immunohistochemistry was performed on twenty-six cutaneous MCT samples. Five random high-power field images (0.08 mm^2 per image) were evaluated for each MCT case. Neoplastic mast cells immunostaining was assessed regarding both the percentage of positive cells and staining intensity. Positive tumour-infiltrating lymphocytes (TILs) were counted. The results were compared with histological grades and post-surgical survival.

Results
CD30 positivity in neoplastic mast cells averaged 92.47% (47.33%-100%) and varied in intensity, being strong (n=18) or weak (n=8). CD30-positive TILs count averaged 23 (range: 1-79). Kaplan-Meier analysis revealed shorter survival for dogs with neoplastic mast cells presenting strong immunostaining intensity (p=0.0171). A cut-off of 38 CD30-positive TILs per sample was established using a ROC curve. Dogs with CD30-positive TIL count higher than 38 had shorter post-surgical survival (p=0.0057).

Conclusion
Our preliminary results suggest that CD30 is a potential prognostic marker for MCTs.

POSTER ABSTRACTS

Veterinary Pathology: Small Animal

185 | CANINE INFLAMMATORY MAMMARY CARCINOMAS EXPRESS HIGHER E-CADHERIN LEVELS THAN NON-INFLAMMATORY CARCINOMAS

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Background
Inflammatory mammary carcinomas (IMCs) are characterised by rapid growth and high metastatic rates in both dogs and humans. E-cadherin downregulation has been associated with poor prognosis in canine mammary tumours. However, in women, E-cadherin is overexpressed in inflammatory breast cancer. The purpose of this study was to compare E-cadherin expression in IMCs with non-inflammatory mammary carcinomas (NIMCs) in bitches.

Materials & Methods
Twenty-five cases of NIMCs and 33 of IMCs were submitted to immunohistochemistry to detect E-cadherin. Immunostaining was evaluated regarding the percentage of positive cells as: 0 (‹25%), 1 (25-50%), 2 (›50-75%) or 3 (›75%). The localization of the protein was recorded as cytoplasmic, membranous or both. Histological subtypes were grouped into G1 or less aggressive tumours (tubular, tubulopapillary, complex and mixed-type carcinomas), and G2 or highly aggressive tumours (solid, micropapillary, anaplastic and comedocarcinomas) according to previously published results.

Results
The percentage of E-cadherin-positive cells in IMCs was higher than in NIMCs (p=0.0003). The majority of IMCs showed cytoplasmic or membranous and cytoplasmic labelling for E-cadherin, while most NIMCs showed cytoplasmic expression (p=0.0188). Most of the IMCs were G2 carcinomas, while the majority of NIMCs were G1 (p=0.0004). However, no differences were found between the histological subtypes regarding the percentage and location of E-cadherin expression.

Conclusion
Canine IMCs present higher levels of E-cadherin expression than NIMCs, similarly to inflammatory breast cancers in humans.

POSTER ABSTRACTS

Veterinary Pathology: Small Animal

186 | THREE CONCURRENT OVARIAN NEOPLASMS IN A FEMALE GOLDEN RETRIEVER DOG

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Background
Ovarian tumors are frequently described in adult female dogs and are noted due to behavioral changes, lactation and vaginal discharge. Usually, ovarian neoplasms grow as a single subtype, either monolaterally or less often bilaterally. Neoplasms of the ovary can be classified into four categories: sex cord-stromal, germ cell, epithelial and mesenchymal tumors.

Materials & Methods
A 12 year-old female golden retriever dog underwent ovariohysterectomy due to pyometra. Both ovaries and uterus were formalin-fixed and submitted for histopathological examination. Immunohistochemistry was also performed on the ovary.

Results
At histology, three different tumors were diagnosed in the right ovary. One tumor showed a cystic-papillary exophytic and endophytic benign growth, occupying 20% of the section. This papillary adenoma (PAP) was multifocally colliding with a second neoplasm (40% of the section) showing sheets of round cells with moderate anisocytosis and anisokaryosis (dysgerminoma - D). Colliding with both and combined with the PAP, a third neoplasia (20% of the section in total) of oval to elongated cells forming nests and tubules, focally surrounding microcavities of eosinophilic amorphous material (Call-Exner bodies) (granulosa cell tumor - GCT) was noted. PanCK was strongly and diffusely expressed in PAP and moderately and diffusely in GCT. Vimentin was strongly and diffusely expressed in the three neoplasms. HER-2 was weak and diffuse in PAP and GCT. Desmin was multifocally detected in PAP and D.

Conclusion
To our knowledge, this is the first case of three concurrent tumors in the ovary of dog.

POSTER ABSTRACTS

Veterinary Pathology: Small Animal

189 | EGFR EXPRESSION IN INFLAMMATORY AND NON-INFLAMMATORY CANINE MAMMARY CARCINOMAS

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Background
Mammary gland tumours are frequent in bitches, and inflammatory mammary carcinoma (IMC) stands out for malignancy. The EGFR is a transmembrane tyrosine kinase receptor whose expression has been associated with more aggressive mammary tumours. This study aimed to evaluate the EGFR expression in IMCs and non-inflammatory mammary carcinomas (NIMCs).

Materials & Methods
Fifty cases of NIMCs and 33 of IMCs were submitted to immunohistochemistry to detect EGFR. Five images from hotspots were captured from each tumour. The percentage of the positive cells was graded as 1 (1–10% positive cells), 2 (11–50%), 3 (51–80%) or 4 (›80%); and the staining intensity as 0 (negative), 1 (weak), 2 (moderate) or 3
(strong). The values were summed up and a final score was considered as low-EGFR (‹5) and high-EGFR expression (6-7).

Results
The percentage of EGFR-positive cells in IMCs (96.03 ± 6.93%) was higher than in NIMC (88.92 ± 16.11%; p=0.0192). Using the combined scores, we found that a higher proportion of the IMCs showed high-EGFR expression when compared with NIMCs (p=0.0387).

Conclusion
Our preliminary results suggest that EGFR is overexpressed in more aggressive carcinomas, especially in IMCs. Complementary studies are needed to investigate EGFR immunohistochemical detection as a potential prognostic and/or predictive marker for the disease.

POSTER ABSTRACTS

Veterinary Pathology: Small Animal

190 | COMPARATIVE ANALYSIS OF CD117/KIT EXPRESSION AND PROLIFERATION INDEX IN CANINE INFLAMMATORY AND NON-INFLAMMATORY MAMMARY CARCINOMAS

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Background
Inflammatory mammary carcinoma (IMC) represents the most aggressive form of mammary tumours in dogs and humans. CD117/KIT (stem cell factor receptor) is a tyrosine kinase growth factor receptor that is the product of the KIT gene. Cancer stem cells have been identified across several neoplasms, with CD117 recognized as a stem cell marker expressed in aggressive tumours. However, the expression of CD117 in IMCs has not been previously examined. This study aimed to evaluate the expression of CD117 in aggressive canine mammary tumours and whether CD117 expression plays a role in the “inflammatory phenotype” and neoplastic growth.

Materials & Methods
Samples of twenty-four grade III non-inflammatory mammary carcinomas (NIMC) and 32 IMC cases were submitted to immunohistochemistry to detect CD117 and to determine the proliferation index using Ki67. The entire tumour area was analysed on scanned slides to determine the percentage of CD117 labelling, as well as the intensity and location of expression. Ki67 index was evaluated according to previously established methodology.

Results
Moderate intensity of CD117 labelling was observed in 50% of the IMCs and 52% of the NIMCs, with predominance of cytoplasmic staining in both groups. The Ki67 index was significantly higher in IMCs (34.96%) compared with NIMCs (24.05%; p=0.011). CD117 expression was not associated with Ki67 proliferation index.

Conclusion
These findings suggest that CD117 expression is high in aggressive canine mammary cancer without a special role on the “inflammatory phenotype” and tumour proliferation. These results provide a scientific basis for targeted therapy with tyrosine kinase inhibitors.

POSTER ABSTRACTS

Veterinary Pathology: Small Animal

191 | EXTRAMEDULLARY PLASMACYTOMA OF THE PANCREAS IN A DOG WITH RENAL AMYLOIDOSIS

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Background
Extramedullary plasmacytomas involving the pancreas have been rarely reported in human literature, and there have been no documented cases in veterinary literature. Here, we present a case of pancreatic plasmacytoma with hepatic involvement, accompanied by renal amyloidosis, in an 11-year-old Terrier with underlying valvular endocardiosis.

Materials & Methods
Clinical assessment was conducted using blood analysis, radiography, and ultrasonography. Due to the grave prognosis, euthanasia was performed, and all tissues were submitted for histopathological diagnosis.

Results
The blood analysis showed no abnormalities, and the bone marrow biopsy revealed normal cellularity without plasmacytosis. Ultrasonography revealed a well-defined, hypoechoic mass in the pancreas, as well as several hypoechoic lesions in the liver. A well-circumscribed, unencapsulated mass measuring 15 mm x 14 mm x 14 mm was found in the pancreas, along with multiple pale areas on the cut surface of the liver. There was no evidence of distant metastasis. Histopathologically, the pancreatic neoplasm consisted of dense sheets of neoplastic round cells characterized by eccentric round nuclei, coarsely-stippled nuclear chromatin, and eosinophilic to amphophilic cytoplasm, supported by fibrovascular stroma. Multifocal infiltration of neoplastic cells were seen in hepatic parenchyma. Immunohistochemistry showed that these cells were immunoreactive for MUM-1 and non-immunoreactive for CD20. Additionally, renal tissues exhibited acellular congo red-positive deposition of amyloid within the majority of glomeruli.

Conclusion
Based on pathological findings, a diagnosis of pancreatic plasmacytoma with renal amyloidosis was made. To the best of our knowledge, this is the first reported case of extramedullary plasmacytoma of the pancreas with concurrent renal amyloidosis in dogs.

POSTER ABSTRACTS

Veterinary Pathology: Small Animal

198 | COMPARATIVE ANALYSIS OF THE IMMUNOHISTOCHEMISTRY-BASED MOLECULAR CLASSIFICATION AND KI67 PROLIFERATION INDEX OF CANINE, FELINE AND HUMAN MAMMARY GLAND TUMORS

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Background

Canine and feline mammary tumors (CMTs and FMTs) are relevant models for translational oncology. The immunohistochemistry-based molecular classification in human breast cancer (HBC) has provided a framework for prognosis and prediction. However, the molecular subtypes of CMTs and FMTs and the similarities with HBC has not yet been well established. The aim of this study was to compare CMTs and FMTs and HBCs based on molecular subtypes and their proliferation index.

Materials & Methods

CMTs (n=58), FMTs (n=53) and HBC cohorts (n=56) were classified into the molecular subtypes (Luminal A, Luminal B, HER2 and triple negative (TN) subtypes) by immunohistochemistry according to two classifications (Nielsen et al., 2004 and Abadie et al., 2017). Ki67 proliferation index was also determined.

Results

In the Nielsen classification, higher percentages of Luminal A cases (36% in CMTs, 53% in FMTs and 41% in HBC) and similar proportion of HER2 cases were observed in all cohorts. However, the TN subtype was more prevalent in CMTs (36%) and FMTs (27%) than in HBCs (7%). The Abadie classification showed higher percentage of Luminal B cases in FMTs (46%) in compared to CMTs (12%). Regarding Ki67 index, TN subtypes showed highest ki67 index in HBCs and FMTs except for CMTs that was observed in HER2 cases.

Conclusion

Our findings identify many similarities between dogs and cats and women across the main molecular subtypes, but also some important differences. These results emphasize the need to stratify into molecular subtypes when considering dogs and cats as models for translational oncology.

POSTER ABSTRACTS

Veterinary Pathology: Small Animal

199 | GENDER-SPECIFIC CANCER SUSCEPTIBILITY IN DOGS: INSIGHTS FROM THE SWISS CANINE CANCER REGISTRY (2008-2020)

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Background
Cancer is common in dogs and is considered one of the leading causes of death. However, gender-specific differences in susceptibility to individual cancer types are poorly characterized in this species.

Materials & Methods
In a historical cohort study, we determined cancer incidence rates (IR) by sex per 100’000 dog-years at risk in the Swiss dog population based on 24’076 cases diagnosed histologically and cytologically between 2008 and 2020. Tumors were coded using the Vet-ICD-O-canine-1.

Results
The overall cancer IR was 338 (female: 386, male: 280), with the highest IRs at 11 years of age in both females and males (IR: 1’000 vs. 698, respectively). Females had higher IRs at all ages, except at the age of 2 years (female: 26, male: 33). Among the five most commonly diagnosed cancers, we found higher IRs in females for mast cell tumors (MCT) (n=5’015; IR: 80 vs. 59), adenocarcinomas (n=2’666; IR: 52 vs. 22), and soft tissue sarcomas (STS) (n=1’743; IR: 25 vs. 23). Male dogs were more frequently affected by lymphomas (n=2’585; IR: 38 vs. 34) and melanomas (n=1’744; IR: 26 vs. 22) than females. Breeds with the highest IRs for the above cancers were female Manchester Terriers (IR: 804), Field Spaniels (IR: 725), and Czechoslovakian Wolfhounds (IR: 167); and male Picardy Sheepdogs (IR: 254) and King Charles
Spaniels (IR: 154), respectively.

Conclusion
Analysis of this large data set suggests gender-specific differences in cancer susceptibility in dogs. Further research into the underlying mechanisms of sex-related cancer susceptibility is warranted.

POSTER ABSTRACTS

Veterinary Pathology: Small Animal

207 | DIFFERENTIAL DIAGNOSTIC CHALLENGE OF A CRANIAL MEDIASTINAL MASS INVOLVING THE LUNG IN A DOG

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Background
Mediastinal masses in dogs are unusual congenital defects which mainly arise from the thymus, bronchi, ectopic thyroid tissue, remnants of the branchial pouches or lung congenital anomalies.
This work describes the pathological and immunohistochemical findings of a surgically removed mediastinal mass (7x6 cm in diameter) in a 9-year-old female Brittany-Spaniel dog suffering from respiratory distress and exercise intolerance.

Materials & Methods
Multiple samples were routinely processed for histopathology and immunohistochemistry (eight cytokeratins (CKs), vimentin, muscle specific actin and Ki-67).

Results
The mass presented irregular surface, surrounded by a thick capsule, a lung fragment and lymph node. At section, the mass showed heterogeneous pattern with an extensive central friable area. Histologically, numerous, variable size, thin-walled cysts with papillae growth were lined by epithelium from cuboidal to columnar or pseudostratified ciliated, that was AE1/AE3+, MNF116+, CK5/6+, CK7+, CK18+, CK19+, mild CK8+ and focally CK20+. Moreover, proliferation of neoplastic epithelial cells disposed in solid or trabecular pattern and infiltrative growth expressed MNF116+ and CK5/6+, mild AE1/AE3+. In them, areas with acinar differentiation were positive for CK7+, CK8+ and CK19+. Ki67+ index was low. In the centre of the mass, large amount of fibrin, necrotic debris and cholesterol clefts surrounded by granulation tissue were observed. The lung fragment was apparently normal. Neoplastic cells (AE1/AE3+) were found in lymph node.

Conclusion
Differential diagnosis presented a challenge. Based on the above-mentioned features suggesting pulmonary differentiation, diagnoses could be consistent with a branchial cyst with basaloid carcinomatous transformation or a lung hamartoma with carcinomatous evolution.

POSTER ABSTRACTS

Veterinary Pathology: Small Animal

215 | IDENTIFICATION OF DISTINCT SIGNALING PATHWAYS IN THE MOLECULAR SUBTYPES OF CANINE MAMMARY CARCINOMAS

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Background
Molecular subtypes of canine mammary tumours (CMTs) have shown considerable similarity to human tumours at both histopathological and transcriptional levels. However, the molecular landscape that defines the different molecular subtypes in CMTs is still poorly understood. The aim of this study was to assess the gene expression profile of a CMTs cohort to provide distinct molecular signatures and to identify specifically altered pathways in comparison with normal mammary glands.

Materials & Methods
Eight canine mammary carcinomas were classified by immunohistochemistry into the molecular subtypes (Luminal A, Luminal B, HER2 and triple negative (TN) subtypes). Moreover, RNA from each tumour and two healthy mammary glands was isolated using the RNAeasy Isolation Kit (Qiagen) following the manufacturer´s protocols. Differential expression analysis was performed by RNA-seq and the most significantly differentially expressed genes (DEGs) between groups with thresholds shrinked FC>2 and Benjamini-Hochberg adjusted p value <0.1 were determined.

Results
We identified specific signalling pathways dominant in each molecular subtype including androgen receptor signature within the TN subtype, reactive oxygen species pathways and glycolysis for Luminal A and oxidative phosphorylation pathway for Luminal B subtype. Additionally, specific clusters of genes were upregulated (FADS1, MERTK, SF3B2, TTC21A and GBF1 genes) and downregulated (PGM3, PIGM, GAPAA1, ARSB and PCBP2 genes) for TN subtype.

Conclusion
Our findings suggest the existence of molecular networks for TN subtype, characterized by explicit alterations in the androgen receptor and metabolic pathways and identify novel insights into the molecular subtypes of CMTs which add clarity to the nature of this entity.

POSTER ABSTRACTS

Veterinary Pathology: Small Animal

228 | A CASE OF AMELOBLASTOMA IN DOMESTIC SHORTHAIR CAT

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Background
Solid masses such as ameloblastomas are not common in the sinuses of cats. Ameloblastoma is a slow-growing tumor of odontogenic epithelial origin that does not contain odontogenic ectomesenchyme. The tumor is non-metastatic but locally aggressive with a high risk of recurrence.

Materials & Methods
A 13 year-old domestic hypersalivating European shorthair cat was brought into the clinic repeatedly due to a swelling under the left eye, located at the cheekbone area, with purulent discharge. A large hard intraosseal/intrasinusal mass with invasion of underlying and surrounding anatomical structures (maxilla and cheekbone) was observed. After 2 months, radiological examination showed significant progression of bone lysis and decreased tissue density in both maxilla and cheekbone. Routine HE staining and immunohistochemical stainings for BRAF V600E and calretinin were performed.\

Results
On HE examination there were sheets and cords of anastomosing odontogenic epithelial cells supported by fibrovascular connective tissue stroma. A peripheral palisading arrangement and reverse polarity, basilar epithelial cytoplasmic clearing of epithelial cells were observed. Also, non-basilar (central acanthocytes) epithelial connection by prominent intercellular bridges was noticed. The central cells were polygonal, with large, unevenly sized, round nuclei with a single nucleolus. Anisocytosis and anisokaryosis were mild. Variable keratinization with indistinct keratin pearls were seldom visible. The mitotic count per 10 hpf was 22 mitosis. The IHC results showed strong positivity for BRAF V600E in basilar and central acanthocytes. Calretinin was negative.

Conclusion
The final diagnosis was ameloblastoma and treatment with Zelboraf was started. Three days later animal died unexpectedly.

POSTER ABSTRACTS

Veterinary Pathology: Small Animal

229 | CANINE INFLAMMATORY MAMMARY CANCER: INVESTIGATING THE TRUE MEANING OF “INFLAMMATORY”

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Background
Canine inflammatory mammary cancer (IMC) is a very rare type of CMC and the most aggressive form, often misdiagnosed as mastitis due to its inflammatory-like clinical features (heat, swelling, erythema, hardening, pain). It is generally accepted that these clinical characteristics of inflammation do not have a histopathological correlation, as IMC does not show greater inflammatory infiltration than other CMC types on H&E slides, leading pathologists to dislike the term ‘inflammatory. Aimed at discovering if this latter affirmation is true, this study compares inflammatory infiltration in the tumor microenvironment (TME) of IMC and non-IMC CMC cases.

Materials & Methods
Eight cases of dogs with IMC and eight non-IMC CMCs of grades II (intermediate) and III (high) were prospectively selected for a retrospective study. The inflammatory infiltration of the TME was assessed using immunohistochemistry for CD3 (T lymphocytes), CD20 (B lymphocytes), FoxP3 (regulatory T cells), Iba1 (macrophages), myeloperoxidase (neutrophils), cKit (mast cells), and MUM1 (plasma cells). The number of cells per mm² was quantified and statistically compared between groups (IMC vs non-IMC).

Results
The number of B lymphocytes, T lymphocytes, regulatory T cells, and neutrophils was significantly higher in IMC compared to non-IMC ($p \leq 0.01$ for all comparisons). No significant differences were observed in mast cells, macrophages, and plasma cells between the groups.

Conclusion
This study reveals that there is indeed a higher level of inflammation at the histopathological level in IMC, which explains, to some extent, the distinctive clinical features of this type of mammary cancer.

POSTER ABSTRACTS

Veterinary Pathology: Small Animal

230 | ADDING A NEW DIMENSION TO FELINE HYPERTROPHIC CARDIOMYOPATHY: A 3D APPROACH TO INVESTIGATE THE PATHOGENESIS

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Background
Feline hypertrophic cardiomyopathy (fHCM) is the most frequently diagnosed cardiomyopathy in domestic cats. Previous studies have indicated that it associates with a reduction in contractile mass and microvascular density and an increase in interstitial connective tissue. We have now explored the benefit of three-dimensional reconstructions to further investigate these myocardial changes.

Materials & Methods
A series of 40 consecutive sections (5 µm) from approximately 8 x 15 mm tissue specimens of the left ventricular free wall from each four cats with fHCM and normal controls were stained by immunohistochemistry for the endothelial cell marker CD31, and digitalised. Using the HeteroGenius Medical Image Manager (MIM) software, a convolutional neural network was trained to classify vessels, cell cytoplasm and nuclei, and sections were aligned; a classification mask was subsequently applied. A volume was generated and exported to the 3D Slicer software, for segmentation and quantification of the classified structures.

Results
In the fHCM myocardium, blood vessels were less numerous but had a higher volume and surface area. Nuclei were less numerous and had a smaller mean surface area and significantly lower volume. The total cell volume was significantly lower, whereas the interstitium had a higher volume than in the control hearts.

Conclusion
The three-dimensional approach proves what previous 2D studies have indicated: the fHCM myocardium has a reduced cellularity and vascular density and a wider interstitium. At the same time, the tendency for higher vessel volumes and larger exchange surface suggests attempts at compensation for the relative loss of functional tissue.

POSTER ABSTRACTS

Veterinary Pathology: Small Animal

231 | CONCOMITANT INTESTINAL PERIPHERAL NERVE SHEATH TUMOR (SCHWANNOMA) AND GANGLIONEUROMATOSIS IN A DOG

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Background
Schwannomas are tumors originating from Schwann cells that form the neural sheath. The incidence of intestinal schwannoma is extremely low. Ganglioneuromatosis (GN) is an abnormal, extensive, poorly defined, and generally transmural proliferation of the neural elements of the intestine. The conjunction of these two conditions is a unique finding, and the relation between them should be studied

Materials & Methods
An 8-year-old male “Villano de las Encartaciones” dog was presented with anorexia and weight loss. Abdominal ultrasonography revealed an obstructive ileocecal intussusception with segmental circumferentially thickening obliterating its lumen. Exploratory laparotomy was undertaken and partial ileocaecal excision. The removed intestinal section was analyzed by histological studies.

Results
Histologically, infiltrating and expanding the intestinal submucosa, a proliferation of well-differentiated ganglion cells arranged in aggregates or individually was observed. Among these cells, expanding and effacing the lamina propria, variably arranged in storiform patterns and interlacing streams, a proliferation of neoplastic spindle cells was observed. The immunohistochemical studies revealed a strong positivity in all these cells against the markers S-100, GFAP, vimentin and negativity against α-sma. These findings were consistent with a diffuse GN together with the presence of an intestinal schwannoma.

Conclusion
Diffuse GN is a change of non-neoplastic nature and benign behavior, whose etiology is not entirely clear, although it is assigned a congenital origin. In this case, the concomitant presence of a peripheral nerve sheath neoplasm could suggest a synergistic relationship between both processes. Both conditions should be included in the differential diagnosis in dogs with intestinal thickening.

POSTER ABSTRACTS

Veterinary Pathology: Small Animal

232 | DECIPHERING VIRAL REPLICATION DYNAMICS IN FELINE INFECTIOUS PERITONITIS: A QUANTITATIVE APPROACH

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Background
Feline infectious peritonitis (FIP) is a complex disease with diverse clinical manifestations and outcomes. Despite advancements in understanding its cause, pathogenesis and treatment, challenges persist in elucidating viral factors related to virion structure and replication of the causative agent, feline coronavirus (FCoV). This study explored
FCoV infected-cats with and without FIP for potential associations or variations in expression ratios of different viral genes.

Materials & Methods
We designed RT-qPCR assays with an RNA based standard targeting the mRNA or gRNA of FCoV envelope and polymerase genes. RNA was extracted from lesioned tissue samples of 50 cats diagnosed with FIP based on pathological examination and immunohistology for FCoV, and mesenteric lymph nodes of 10 cats with PCR confirmed FCoV infection but no FIP, to comparatively assess variations in viral gene expression ratios.

Results
In FIP cats, the polymerase mRNA assay yielded the highest copy numbers, followed by the polymerase combined mRNA/gRNA assay; the envelope mRNA assay yielded the lowest copy numbers. The statistical analysis indicated a correlation between the assays ($p<0.001$). In cats without FIP, viral gene expression was either not detected (n=6) or at low levels (n=4) in one or more assays. One of the latter cats exhibited viral RNA levels higher than those of some FIP cats.

Conclusion
The results suggest consistent virion production in association with FIP lesions but not with FCoV infection alone. Future studies should explore whether monitoring of viral and in particular polymerase mRNA levels could potentially guide efficient treatment strategies.

POSTER ABSTRACTS

Veterinary Pathology: Small Animal

234 | DEPOSITION OF PROTEINACEOUS PAS-POSITIVE MATERIAL IN A CASE OF CANINE B CELL LYMPHOMA

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Background
Accumulation of periodic-acid Schiff (PAS) positive, proteinaceous material in the lymph nodes of humans with B cell lymphoproliferative disorders has previously been reported. This material is distinct from fibrin, amyloid and collagen and may derive from exocytosis of overproduced cell membranes. We report a case akin to this condition in a 3-year-old, male, mixed breed dog that presented with abdominal pain and lymphadenomegaly of the medial iliac lymph node on ultrasound.

Materials & Methods
Flow cytometry was conducted on single cell suspensions of the medial iliac lymph node using the following antibodies: CD3, CD4, CD8, CD21, CD79, CD34, CD5, CD18, and MHCII.
Formalin-fixed and paraffin-embedded samples of the medial iliac lymph node were initially stained with hematoxylin/eosin and PAS. Subsequent additional stains included: Verhoef van Giesson, Alcian blue, Congo red and phosphotungstic-acid hematoxylin.
Immunohistochemical stains were performed at two external laboratories. Antibodies tested included: Pax-5, Mum 1, CD79a, CD20 and CD3.

Results
Histology revealed massive accumulation of amorphous, PAS-positive material associated with a large cell lymphoma. Immunohistochemical tests and flow cytometry confirmed a B cell lymphoma (diffuse large B cell lymphoma).
The PAS-positive material was negative to all aforementioned additional histochemical stains.

Conclusion
The presence of abundant PAS positive material in this canine B cell lymphoma is similar to what is described in human patients with lymphoproliferative B cell disorders. Additional techniques like transmission electron microscopy could further elucidate the composition of this material. The clinicopathological and prognostic relevance of PAS-positive material deposition in canine lymphomas requires further investigation.

POSTER ABSTRACTS

Veterinary Pathology: Small Animal

235 | HISTOPATHOLOGY AND IMMUNOLABELLING OF THE VIRAL ANTIGEN IN THE TISSUES FROM CATS INFECTED WITH A/H5N1 AVIAN INFLUENZA VIRUS

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Background
In 2023, an outbreak of highly pathogenic avian influenza (HPAI) A (H5N1) virus clade 2.3.4.4b previously confirmed in birds was reported in 34 cats in Poland. This study aimed to assess the histopathological changes and the localisation of viral antigen using immunohistochemistry in selected tissues collected from four of the cats which died and were tested positive for the virus by real-time RT PCR.

Materials & Methods
Formalin-fixed paraffin-embedded tissue sections from brain, lungs, liver, pancreas, jejunum, heart and kidney from the cats (n=4) were subjected to HE staining and immunohistochemistry using mouse monoclonal antibody anti influenza A nucleoprotein (HYB 340-05) and a commercial HRP/DAB+ staining kit.

Results
Histopathological examination of the brain revealed moderate multifocal gliosis and perivascular lymphocytic infiltrates. In the lungs moderate interstitial pneumonia to moderate lymphohistiocytic bronchopneumonia was observed. In one liver there were multifocal necrotic foci with aggregates of lymphoid cells and mild perivascular lymphohistiocytic infiltrates. The viral antigen immunolabelling was visible in the neural and glial cells in all the brain tissues and in the inflammatory infiltrations in all the lungs and one liver. No distinct histopatological changes and no immunolabelling were observed in pancreas, jejunum, heart or kidney.

Conclusion
The detected HPAIV variant showed predilection mainly for nervous tissue and respiratory tract in the examined cats, indicating that respiratory exudate might be the main source of the virus shedding. Although direct transmission from cats to humans was not reported, the results confirmed the importance of continuous surveillance of the zoonotic influenza.

POSTER ABSTRACTS

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243 | DISSEMINATED SCLEROSING EPITHELIOID MESOTHELIOMA RESEMBLING SCIRRHOUS ANAPLASTIC CARCINOMA

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Background
Mesotheliomas are neoplasms that arise from mesothelial cells lining the coelomic cavities. The differentiation of atypical forms of mesotheliomas and metastatic carcinomas may be difficult histologically, mainly when the epithelial tumor is highly anaplastic and undifferentiated

Materials & Methods
A 6-year-old male German Shepherd dog presented with progressive weight loss and abdominal pain. Imaging tests reveal abdominal and thoracic effusion and diffuse micronodular lesions in thorax and abdominal cavity.
A cytological examination of the effusion showed reactive mesothelial cells and numerous epithelioid and fusiform cells with atypia. The morphology of the neoplasm cells guided the presumptive diagnosis towards metastatic carcinoma. Due to the poor prognosis, the owners elected euthanasia

Results
At the necropsy, multifocal to coalescing 0.3 and 0.4 cm white, firm nodules was present on all abdominal serosal surfaces, pleura, pericardium and tunica vaginalis. Histologically, nodules were composed by neoplastic cells were loosely arranged in clusters and were frequently individualized and supported by abundant coarse fibrous stroma. The absence of involvement of parenchymal organs as well as the strong immunopositivity of the neoplastic cells against cytokeratin and vimentin guided the diagnosis towards an atypical sclerosing mesothelioma

Conclusion
The morphological similarity of neoplastic cells in atypical sclerosing mesotheliomas and scirrhous anaplastic carcinomas, as well as the co-expression in both processes of immunohistochemical markers such as vimentin and cytokeratin, pose a diagnostic challenge in the differentiation of this type of neoplasms, which is important in the treatment and prognosis of these conditions.

POSTER ABSTRACTS

Veterinary Pathology: Small Animal

248 | MULTINUCLEATED GIANT CELLS (MGCS): A PHENOTYPIC ASSESSMENT IN NEOPLASTIC AND NON-NEOPLASTIC LESIONS OF DOGS AND CATS

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Background
Multinucleated giant cells (MGCs) are observed in inflammatory and neoplastic lesions. In granulomatous reactions, macrophages fuse to form large MGCs. In tumors, MGCs may originate from macrophages (tumor-associated inflammation) or neoplastic cells. Despite numerous lesions show these fascinating cells in veterinary medicine, their characterization has been sporadic. This pilot study aimed to investigate the phenotype of MGCs in selected lesions of dogs and cats, encompassing both inflammatory and neoplastic lesions.

Materials & Methods
For this purpose, various lesions were retrospectively selected from FFPE archive material at the Veterinary Pathology Service of the Department of Veterinary Medicine (Perugia, Italy) based on the presence of MGCs. Seven peripheral giant cell granulomas, 6 feline injection-site sarcomas (FISS), 6 osteosarcomas (OSA), 4 oral melanomas, 2 giant cell tumor of tendon sheath, and 1 granulomatous rhinitis were submitted to IHC using a panel of macrophagic-specific antibodies (IBA1, CD204, CD206, CD163, MAC387), in addition to phenotyping markers specific of the investigated lesion.

Results
In most cases, MGCs showed a strong and diffuse positivity for IBA1, and negativity for the other macrophagic markers and specific antibodies (i.e. RUNX2 in osteosarcomas, Melan-A/PNL2/SOX-10 in melanomas).
Occasional CD206 and MAC387 expression was observed in FISS. In tumors, some MGCs with cellular atypia were negative for macrophagic markers.

Conclusion
MGCs in different inflammatory and neoplastic lesions seem to have a histiocytic origin, although their specific origin and role within the neoplastic microenvironment remain unknown. Intracellular nuclear atypia and negativity to macrophagic markers might help the pathologist distinguish intratumoral MGCs of neoplastic origin from non-neoplastic histiocytic elements.

POSTER ABSTRACTS

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249 | RETROSPECTIVE ANALYSIS OF TUMOUR INCIDENCE AT THE COMPLUTENSE VETERINARY TEACHING HOSPITAL OF MADRID: 2012-2022

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Background
Investigating malignancy data in canine and feline tumours is crucial for clinicians to identify high-risk patients and guide treatment strategies effectively. This study aimed to compare tumour profiles in cats and dogs, considering animal-specific factors such as sex, age, breed, and tumour location. Additionally, the study sought to examine the involvement of lymph nodes in cases of malignant tumours.

Materials & Methods
The study analysed data from biopsies conducted over a 10-year period at the Pathology Service of the Complutense Veterinary Teaching Hospital of Madrid.

Results
A total of 1,595 registries were examined, comprising 1,453 records from dogs (91.0%) and 142 from cats (8.9%). The majority of records (71.2%) were classified as malignant. In dogs, the malignancy proportion (MP) was 69.4%, while in cats, it exceeded 90%. The most frequently diagnosed tumours in both species were found in the mammary gland (30.5% in dogs, 26.8% in cats) and skin and appendages (30.1% in dogs, 26.1% in cats). Furthermore, 19.9% of lymph nodes were assessed in cases of malignant tumours. Metastatic lymph nodes were predominantly associated with digestive tumours in dogs and mammary tumours in cats.

Conclusion
Mammary tumours remained the most diagnosed tumours in both cats and dogs in this area. Malignant tumours were more prevalent among cats. The presence of metastatic lymph nodes was primarily linked to digestive and mammary tumours. Observations from this work indicate a trend of removing a limited number of lymph nodes, highlighting the necessity for a more extensive lymph node dissection, including sentinel node detection.

POSTER ABSTRACTS

Veterinary Pathology: Small Animal

253 | EEEH!? A CASE OF EASTERN EQUINE ENCEPHALITIS IN A DOG

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Background
Eastern equine encephalitis (EEE) virus (Togaviridae) is a vector-borne, transboundary disease in North America. Fatal infections occur in humans and horses; however, the relevance and occurrence of the EEE virus in other domestic animals is underreported.

Materials & Methods
A five-month-old, male intact, Australian Shepherd puppy presented with a history of five-day fever and rapidly progressive neurologic signs. Following magnetic resonance imaging, the puppy was euthanized, and the brain submitted for evaluation to the New York State Animal Health and Diagnostic Center (AHDC), Cornell University. Histologic evaluation and immunohistochemistry for EEE, West Nile virus, canine distemper virus, and rabies virus were performed along with PCR for EEE and sanger sequencing on formalin-fixed paraffin-embedded (FFPE) tissue.

Results
Histologic examination revealed severe, diffuse, subacute, lymphohistiocytic and neutrophilic, necrotizing meningoencephalitis, leptomeningitis, and choroid plexitis with neuronal necrosis, glial nodules, neuronophagia, axonal spheroids, and digestion chambers. Robust EEE virus immunolabelling was observed in the brain and EEE virus infection was confirmed by PCR and sanger sequencing followed by phylogenetic analysis. The case was compared with two previous cases of EEE-positive dogs submitted to AHDC in 2011.

Conclusion
EEE is a fatal condition in young dogs. EEE virus is not present in Europe yet but the arthropod vectors of EEE (Aedes vexans, Coquillettidia perturbans, etc.) are, and some European countries (Belgium, the Netherlands, and Italy) are regarded as hotspots for the introduction of EEE. This report highlights the importance of including EEE in the differential diagnosis of canine meningoencephalitis, even in non-endemic areas.

POSTER ABSTRACTS

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255 | CLINICOPATHOLOGICAL, IMMUNOHISTOCHEMICAL, AND MOLECULAR CHARACTERIZATION OF A CANINE TESTICULAR T-CELL LYMPHOMA

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Background
Canine testicular tumours are common and account for 4 to 7% of all neoplasms in male dogs. Generally, they arise from the sex cord-stromal elements of the gonad or germ cells. Other primary and metastatic tumours are rare, particularly lymphoma. This report aims to describe the morphologic, immunohistochemical, and molecular features of testicular T-cell lymphoma in a dog.

Materials & Methods
A 9-year-old mixed-breed male dog was submitted for clinical evaluation due to apathy, anorexia, and weight loss. At the physical exam, a bilateral asymmetrical testicular swelling was detected, and ultrasound showed an increase in testicular size and diffuse echogenicity alterations. The orchiectomy was performed and the testicles were submitted to histopathological, immunohistochemical, and PARR analysis.

Results
The testicles measured approximately 6×4x3 cm and both at the cut section presented multiple white nodules. Microscopically, it was observed a multifocal neoplastic infiltrate with an intertubular solid growth pattern, which was composed of intermediate-to-large round cells. Neoplastic population strongly expressed CD3 and was negative for PAX5. These features in combination with the results of clonality assessment were consistent with extranodal peripheral T-cell lymphoma of the testicle. The patient had extratesticular involvement during the follow-up period, having appeared multiple cutaneous plaques on the dorsal region that were cytologically confirmed as lymphoma. The deterioration of the clinical condition led the owners to choose euthanasia.

Conclusion
Testicular lymphoma is a rare form of extranodal peripheral T-cell lymphoma in dogs. In this report we provide a detailed characterization of this entity, emphasizing the importance of considering it as a differential diagnosis.

POSTER ABSTRACTS

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257 | ANALYSIS OF PD-1:PD-L1/PD-L2 AXIS IN FELINE MUCINOUS MAMMARY CARCINOMAS

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Background
Feline mammary carcinoma often displays aggressive behavior with limited therapeutic options. Recently, PD-1:PD-L1/PD-L2 axis has become a masterpiece in tumor immunotherapy, with anti-PD-L1 antibodies showing great efficacy in human breast cancer. In this work, we analyzed PD-1, PD-L1 and PD-L2 expression in feline mucinous mammary carcinoma (FMMC), and mutations in the PD-L1 gene.

Materials & Methods
PD-1, PD-L1/2 expression was evaluated in 36 FMMC by immunohistochemistry. Positivity (>1% of cells) was explored in intratumoral (iTILs) and stromal (sTILs) tumor-infiltrating lymphocytes, as well as in tumor cells (TC). Genomic DNA extraction was performed in 7 FMMC and exons of interest in the PD-L1 gene were amplified using PCR. The sequenced fragments were aligned with the ClustalW tool.

Results
PD-1 expression was found in TC, iTILs and sTILs of 13.8%, 72.2% and 91.7% of tumors, while 8.3% of samples did not show expression of any protein. PD-L1 expression was detected in 52.8%, 94.4% and 100% of tumors in TC, iTILs and sTILs, respectively. Regarding the PD-L2 expression, 80.6% and 100% of tumors were positive in TC and in both subgroups of TILs. Finally, DNA sequencing results showed no somatic mutations or rearrangements in the five protein-coding exons of feline PD-L1 gene.

Conclusion
These findings highlight the importance of the PD-1:PD-L1/PD-L2 axis in FMMC, considering the expression levels of the three proteins and the absence of mutations in the PD-L1 gene, supporting the development of checkpoint-blocking therapies targeting the PD-1:PD-L1/PD-L2 axis for the treatment of FMMC.

POSTER ABSTRACTS

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260 | CAN KI-67 IMMUNOSTAINING PATTERN BE A PROGNOSTIC MARKER FOR MAMMARY GLAND TUMOUR IN DOGS?

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Background
In 2021 a study carried out with human samples of squamous epithelial tissue, proposed a new way of evaluating Ki-67, through five different patterns of nuclear positivity. The aim of this study was to apply the Ki-67 pattern of nuclear stain in canine mammary tumours.

Materials & Methods
10 cases of adenoma and 10 cases of carcinoma were selected for Ki-67 immunohistochemical analysis. One hundred nuclei from each sample were classified according to the following standardization: fine or coarse granules randomly distributed (NP1 - early G1); one or two larger aggregates, well defined and centralized (NP2 - late G1); granules and aggregates occupying all or part of the nucleus (NP3 - S); intense and homogeneous staining (NP4 - G2); empty central area and presence of peripheral granules/aggregates (mitosis - prometaphase). Prisma software was used for statistical analysis (ANOVA and T test).

Results
Group NP3 was the predominant Ki-67 pattern in both adenoma and carcinoma, followed by NP2. There was a significant statistical difference among Ki-67 nuclear staining patterns in each tumour group ($p<0.0001$); however, when comparing the adenoma vs. carcinoma, there was no statistical difference for any of the patterns ($p>0.05$).

Conclusion
Through these preliminary results, it was not possible to use standardization of nuclear immunostaining to differentiate adenomas from carcinomas. However, due to the possibility of these patterns represent different phases of the cell cycle, it is possible to further study the tumour dynamics of canine mammary neoplasms.

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267 | MULTIPLE PARAGANGLIOMAS IN TWO BOXER DOGS: MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL FEATURES, AND SDHD GENE ANALYSES

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Background
Paragangliomas (PGL) in dogs are rare neoplasia of chemoreceptor organs associated with multiple endocrine neoplasia. This study aims to present the morphological features, immunohistochemical profile, and screening of succinate dehydrogenase subunit D (SDHD) gene status in two cases of multiple PGLs in dogs.

Materials & Methods
Two boxer dogs were submitted for postmortem examination, a male of 9 years and a female of 12 years, both with a history of multiple tumors. The samples collected during necropsy were further examined by histopathology including IHC assessment of the chromogranin-A (CHr-A), neuro specific enolase (NSE), and S-100, and further submitted for SDHD gene mutation screening of exons 2, 3, and 4.

Results
Multiple tumors of endocrine tissues and glands were identified during autopsy and confirmed histologically in both cases. In both cases, individual neoplastic masses were identified at the bifurcation of the common carotid artery and between the aorta and the pulmonary trunk. Histologically, masses were composed of polygonal cells organized in nests, following the characteristic “Zellballen” pattern. The neoplastic cells showed distinct membranes with finely granular cytoplasm and hyperchromatic nuclei. IHC both cases were intensely stained for NSE and Chr-A and weak staining for S-100. Genetic investigations of exons 2, 3, and 4 of the SDHD have not confirmed particular mutations.

Conclusion
The results emphasize that the genetic basis for the occurrence of paraganglioma in dogs is more complex, and its development may be influenced by mutations in other genes as well.

POSTER ABSTRACTS

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273 | CANINE INFLAMMATORY MAMMARY CARCINOMA: A PORTUGUESE MULTICENTRIC RETROSPECTIVE STUDY

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Background Canine inflammatory mammary carcinoma (CIMC) is an uncommon but highly aggressive mammary tumor (MT) subtype. The aim of this multicentric study was to gather and characterize a consistent number of CIMC from four Portuguese institutions.

Materials & Methods Cases of dogs with MT submitted for histopathology between 2001 and 2022 were retrospectively assessed from the laboratory databases. MT with a diagnosis compatible with CIMC were reviewed and histologically graded (Zappulli et al., 2019). Immunohistochemistry was performed for molecular phenotype classification (Goldhirsch et al., 2013), using antibodies for estrogen/progesterone receptors (ER/PR), HER-2 and Ki-67.

Results A total of 49 cases were diagnosed as CIMC, characterized by the presence of neoplastic embolization of superficial dermal lymphatic vessels, associated with distinctive clinical features. Dogs were mainly mixed breed (n=20;40.8%), with a mean age of 10.86 years. The most frequent histotypes were tubulopapillary (n=16; 32.7%), anaplastic (n=11; 22.5%), micropapillary (n=10; 21.3%), and comedocarcinoma (n=5; 10.6%). Most carcinomas were high-grade (n=43; 87.8%) and all cases with available lymph nodes (LN) (n=26) presented LN metastases. CIMC were classified as Luminal B-like (HER2 negative) (ER and/or PR+;HER2-;Ki67≥20%) (n=32; 68%), Triple Negative (TN) (ER and PR-;HER2-) (n=12; 25.5%), Luminal A-like (ER and/or PR+;HER2-;Ki67‹20%) (n=2; 4.3%) and Luminal B-like (HER2 positive) (ER and/or PR+;HER2+; any Ki67) (n=1; 2.1%).

Conclusion CIMC were generally high-grade carcinomas, with LN involvement at diagnosis and classified as Luminal B-like (highly proliferative) or TN immunophenotypes, both classically associated with poor outcome. Further research is required to identify novel therapeutic targets for CIMC, fostered by the participation of collaborative multicenter networks.

POSTER ABSTRACTS

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278 | CANINE EOSINOPHILIC PULMONARY GRANULOMATOSIS: CLINICAL-PATHOLOGICAL DIAGNOSIS AND PHYSIOPATHOLOGY THEORIES

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Background
Eosinophilic pulmonary granulomatosis (EPG) is a rarely reported disease that has not been completely elucidated yet, and is highly associated with heartworms (Dirofilaria immitis). Gross pathology consists on firm masses replacing the pulmonary parenchyma. Microscopically it is composed by a heterogeneous inflammatory cellularity with macrophages and eosinophils predominance.

Materials & Methods
We describe one case of EPG in a 2 year, intact female, pitbull crossbred dog, that was received by emergency service, presenting respiratory distress and abdominal effusion. Ultrasonographic, thoracic radiography and clinical pathology studies were performed. Later, euthanasia was elected due to poor prognosis. Necropsy and subsequently histopathology and immunohistochemistry (IHC) were preformed.

Results
Imaging studies revealed a radiopaque/hyperechoic mass replacing almost the entire left lung, confirmed in necropsy, which consisted of a firm, lobulated, pale mass, that when cut, exhibited a homogeneous, pale surface, with multifocal necrosis areas. Microscopically, it was densely composed by macrophages (IHC positive for lysozyme and Iba-1), eosinophils (positive for LUNA stain), and random lymphocytes and plasma cells. The same pattern was found on enlarged tracheal lymph nodes. Interestingly, the rest of the tissues had random eosinophilic thrombi (correlated with hypereosinophilia in the blood count) and a nematode larva was identified in the kidney.

Conclusion
The physiopathogenesis of EPG is not certainly known. Commonly, heartworms can cause heart and lung blood vessels occlusion; however, in this condition, it is hypothesized that exacerbated Th2 responses, “anaphylactoid” response or local histiocytic proliferation (neoplasm-like) could explain this pathology process.

POSTER ABSTRACTS

Veterinary Pathology: Small Animal

279 | SURVIVAL ANALYSIS OF FEMALE DOGS AND CATS WITH RARE AND UNCOMMON MALIGNANT MAMMARY TUMORS - PRELIMINARY RESULTS

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Background Mammary tumors (MT) are among the most frequent neoplasia of female dogs and cats. Several malignant histotypes, including special histotypes, invasive micropapillary carcinoma (IMC), anaplastic carcinoma (AC), carcinosarcoma (CSa) and carcinoma and malignant myoepithelioma (CMM) are considered rare or uncommon mammary tumors (RUMT), and frequently associated with poor prognosis. Due to their rarity, the information regarding RUMT in companion animals is scarce. This study aimed to describe clinical outcome of female dogs and cats with RUMT.

Materials & Methods Clinical records of female dogs and queens presented to five veterinary medical centers from Portugal between 2012 and 2023 due to MT were evaluated. Females with RUMT and available clinical information were selected. Animals with chronic diseases or history of previous neoplasia were excluded.

Results Fifty female dogs were evaluated, including 15 CSa (overall survival (OS) 3-47m, MST 10m), 9 adenosquamous carcinomas (OS 5-24m, MST 18m), 7 inflammatory carcinomas (OS 0.5-12m, MST 5m), 6 CMM (OS 3-66 m), 5 IMC (OS 1-8m), 4 AC (OS 7-78m), 3 spindle cell carcinomas (OS 5-6m) and 1 mucinous carcinoma (OS 7m). Sixteen queens were studied, including 8 mucinous carcinoma (OS 0.6-15m, MST 12m), 4 adenosquamous carcinoma (OS 0.6-25m), 2 CSa (OS 1.5-5m), 1 IMC (OS 3.5m) and 1 AC (OS 6m).

Conclusion Female dogs and queens with the same histotype presented differences in survival. Future large scale multicentric prospective studies including clinical, molecular and genetic information are needed to better characterize and to evaluate prognostic factors of RUMT in companion animals.

POSTER ABSTRACTS

Veterinary Pathology: Small Animal

284 | FELINE PILOLEIOMYOSARCOMA: A CASE REPORT

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Background
Smooth muscle neoplasia is a infrequently described mesenchymal neoplasia in domestic animals, and may arise from vascular, deep dermal and arrector pili muscle. Diagnostic is based on malignant criteria, like nuclear atypia, mitotic rate and intratumoral necrosis, associated with neoplastic smooth muscle cells in expansive growth. However, diagnosis may be a challenge without complementary techniques, like immunohistochemistry and special stains.

Materials & Methods
Herein, we describe the clinicopathological and immunohistochemical features of a piloleiomyosarcoma in a 6-year-old Sphinx male cat. The cat presented a 0.8cm skin nodule at the right thoracic wall. After surgical excision, tumor was fixed in 10% formalin and submitted for histopathology. Histochemistry (Masson's trichrome, TM) and immunohistochemistry was performed for calponin, desmin, α-smooth muscle actin (SMA), vimentin and cytokeratin.

Results
Tumor was located within the dermis and composed of fusiform cells, arranged in interwoven bundles. Neoplastic cells had eosinophilic fibrillar cytoplasm, elongated blunt-ended nuclei, and showed marked cellular atypia. Scattered multinucleated neoplastic cells and abundant mitoses were observed. Entrapment of hair follicles and absence of vascular components supported an arrector pili smooth muscle origin. At TM stain, neoplastic cells were purple-red. Neoplastic cells were immunopositive to calponin, desmin, SMA and vimentin. No recurrence or metastases were recorded after six-month follow-up.

Conclusion
The morphological and immunohistochemical characteristics confirmed the diagnosis of piloleiomyosarcoma. Surgical resection was curative. Although rare, this entity should be included in the differential diagnosis of feline spindle cell skin neoplasia.

POSTER ABSTRACTS

Veterinary Pathology: Horses

3 | MYCOBACTERIUM AVIUM SUBSP. AVIUM INFECTION IN HORSES WITH GRANULOMATOUS ENTEROCOLITIS

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Background
Infections with non-tuberculous mycobacteria are rare in horses and only occasionally observed in Central and Eastern European countries. The clinical, pathological, microbiological and molecular findings of horses suffering from *M. avium subsp. avium* (MAA) infection are presented.

Materials & Methods
Two horses of different ages from different stables were euthanised due to incurable chronic diarrhea, weight loss and severe cachexia and submitted for post-mortem examination. Haematology and serum biochemistry, histopathology, microbiology and molecular analyses were performed.

Results
Hematological and biochemical abnormalities included leukocytosis, hypoalbuminemia, and hyperfibrinogenemia. Gross pathological lesions were characterized by chronic enteritis with mesenteric lymphadenopathy in two cases and granulomas of the liver and heart in one case. Microscopically, the lamina propria and submucosa of various sections of the small and large intestine were markedly expanded by large numbers of epithelioid macrophages and lymphocytes with fewer plasma cells and variable numbers of multinucleated giant cells, consistent with noncaseous granulomatous enteritis and thyphlocolitis. Enlarged mesenteric lymph nodes, liver and heart granulomas had similar microscopic appearance. Ziehl-Neelsen staining of smears and tissue samples demonstrated acid-fast bacilli. Culture examination and molecular findings confirmed MAA infection.

Conclusion
The current report indicates that horses become naturally infected with MAA despite their relative resistance to mycobacterial infections. This pathogen should be included in the differential diagnosis and treatment- of chronic resistant diarrhea in horses. Diagnosis of suspected cases requires the implementation of molecular biology methods to fully identify the causative agent due to animal and public health importance.

POSTER ABSTRACTS

Veterinary Pathology: Horses

8 | AN UNUSUAL PRESENTATION OF LAWSONIA INTRACELLULARIS IN A YOUNG FOAL

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Background
A 9-month-old female warmblood foal with a longstanding history of poor weight gain and musculature was submitted to an equine clinic after a recent period of anorexia and lethargy. Ultrasound revealed thickening of the small intestinal wall. After acute onset of neurological signs, the foal experienced breathing difficulties. It was euthanized and submitted for necropsy.

Materials & Methods
Full necropsy was performed. Samples were collected and processed for histology and immunohistochemistry. Sections were routinely stained with HE and immunohistochemistry for the expression of *Lawsonia intracellularis* antigen.

Results
The changes in the small intestine were rather indistinct and mainly targeting the duodenum and the proximal jejunum. Grossly, the ileum, caecum and colon were not evidently affected. There was very subtle thickening of the small intestinal wall with inconspicuous irregular erosions and enlarged Peyer's patches. The mesenteric lymph nodes were enlarged. Both kidneys were enlarged and paler. Histologically, the intestinal crypts were not evidently tortuous. There was minimal hyperplasia, with occasional piling up of hyperchromatic epithelial cells and scattered crypt abscesses. Lymphocytes and plasma cells infiltrated the lamina propria and the base of the crypts. *L. intracellularis* antigen was expressed at the level of the crypts as well as in mononuclear cells.

Conclusion
Based on the clinical history, histological and immunohistochemical findings, the diagnosis of *L. intracellularis* was made in this 9-month-old foal. The proximal small intestine was mostly affected, however, the presentation was atypical, as the lesions were subtly erosive and lacked the unique features of classical equine proliferative enteritis.

POSTER ABSTRACTS

Veterinary Pathology: Horses

64 | SPECTRUM OF HISTOLOGIC FINDINGS IN THE LARGE INTESTINE OF CLINICALLY HEALTHY HORSES

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Background
In horses, resident helminth population and gut microbiota can coexist in harmony in the host intestinal ecosystem, but the local tissue response triggered in disease remains unclear. This study provides a baseline for the common histologic findings seen in the large intestine of healthy horses.

Materials & Methods
Microscopic sections from the caecum and dorsal and ventral colon collected from 12 clinically healthy horses at slaughter were evaluated by HE for common changes, neutrophils, eosinophils and mast cells.
These immune cells were counted in 1 mm^2, as were T-cells, B-cells, Treg-cells and macrophages labelled by IHC using CD3, CD79a, FoxP3 and Iba1 antibodies.

Results
In the mucosa on average 4054 (±675.86 StDev) cells were found and the main three cell types were T-cells (40% ± 11.4), diffusely distributed, macrophages (30% ± 9.8), mainly subepithelial clusters, and eosinophils (18% ± 7).
The immune cell composition changed in the submucosa, 2544 (±1185 StDev), with eosinophils (30% ± 14), B -cells (29% ± 10) and macrophages (21% ±9.8) mainly diffusely distributed. Six horses had goblet cell hyperplasia associated with a shift from mainly acidic to mixed and neutral mucin. Marked lymphoid hyperplasia was seen in three horses, of which two had a high number of encysted cyathostomin larvae. Only single larvae were seen in the remaining 10 horses.

Conclusion
This study confirms relatively constant ratios of immune cells. High variability in histologic findings unrelated to the presence of cyathostomin larvae and compartment are features of the large intestine of clinically healthy horses.

POSTER ABSTRACTS

Veterinary Pathology: Horses

119 | FATAL SUXIBUZONE TOXICOSIS IN A SHETLAND PONY

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Background
Suxibuzone is a nonsteroidal anti-inflammatory drug, widely used in equine medicine, particularly for musculoskeletal inflammatory disorders. Upon administration, it is metabolized into the active metabolite phenylbutazone. Despite its therapeutic benefits, suxibuzone carries potential toxicity risks to organs, including the gastrointestinal system, kidneys, and liver, particularly with incorrect dosage or prolonged use. This case documents fatal overdose-induced toxicity resulting from oral administration of suxibuzone in a Shetland pony.

Materials & Methods
A 9-year-old Shetland pony (88 kg), was presented with reduced appetite and weight loss. Blood analyses revealed no significant abnormalities. Subsequently, a treatment regimen of 25,57 mg/kg of suxibuzone daily for 10 consecutive days was initiated (recommended dose 6,25 mg/kg for two days and 3,125 mg/kg thereafter). After 7 days, the pony was found unresponsive, ultimately succumbing shortly thereafter. Necropsy was performed with subsequent histological analysis of multiple organs, including kidney, liver, lung, skeletal muscles, brain, and heart.

Results
Macroscopic examination revealed dark striations evident in both kidneys. Additionally, the teeth exhibited multiple enamel cusps, resulting in severe ulcerations within the buccal mucosae, explaining the clinical complaint of reduced appetite. Histological assessment unveiled acute necrosis affecting renal papillae and tubules, accompanied by the presence of extensive hemoglobin casts. Furthermore, the liver exhibited significant periportal degeneration. No abnormalities were observed in other organs.

Conclusion
Clinical history, in conjunction with histological findings, strongly indicate fatal suxibuzone toxicity at a dosage exceeding four times the recommended level. This case highlights the first documented spontaneous death due to suxibuzone toxicosis in a pony.

POSTER ABSTRACTS

Veterinary Pathology: Horses

130 | INTESTINAL HISTOPLASMOSIS IN A SHETLAND PONY: CASE REPORT AND LITERATURE REVIEW

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Background
Histoplasmosis is an infectious disease caused by *Histoplasma capsulatum*. The classic form of histoplasmosis causes respiratory disease, while the disseminated form is associated with immunosuppression. There are few reports of intestinal histoplasmosis in domestic animals, and they are even scarcer in equines. In this case report, we describe an 11-year-old Shetland Pony with chronic diarrhea and non-respiratory clinical signs. Due to the lack of response to treatment, euthanasia was performed, and a necropsy was conducted.

Materials & Methods
At necropsy, severe granulomatous enterocolitis and lymphadenitis were observed. Representative samples of intestinal and lymph node lesions were evaluated with H&E and Gomori-Grocott stains. Transmission Electron Microscopy (TEM) was performed on paraffin-embedded tissue, which was dewaxed, rehydrated, and fixed in 2.5% glutaraldehyde and 4% paraformaldehyde. PCR for the internal transcribed spacer (ITS) 1-2 ribosomal RNA genes was performed on samples from formalin-fixed sections of intestines. The 550 bp PCR product was purified and sequenced. The sequence was submitted for a BLAST search at the National Center for Biotechnology Information.

Results
Evaluation with H&E and Gomori-Grocott revealed intrahistiocytic yeasts (2–4 µm in diameter), surrounded by a clear capsule. Ultrafine sections were characterized by a thin wall surrounded by a slightly electron-dense capsule, moderate cytoplasm, and a central nucleus. Amplification of the ITS and sequencing identified *Histoplasma capsulatum* (96.4% identity).

Conclusion
There are few confirmed cases of intestinal histoplasmosis in domestic animals. This is the first report of intestinal histoplasmosis in a Pony, and the first one molecularly identified in Mexico.

POSTER ABSTRACTS

Veterinary Pathology: Exotic, wildlife & zoo animals

21 | UNUSUAL PRESENTATION OF SEPTICAEMIC POXVIRUS INFECTION IN HOUBARA BUSTARDS

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Background
Avipoxvirus infections are described in multiple species. Disease may manifest as cutaneous, diphtheritic, or septicaemic. In houbara bustard (*Chlamydotis macqueenii* and *Chlamydotis undulata*) septicaemic form follows an unusual presentation compared to other avian species.

Materials & Methods
Seven houbara with septicaemic poxvirus infection were selected for the study. The birds died in different years and conservation captive breeding centres; Morocco (2015), UAE (2016) and Kazakhstan (2017). Complete necropsies and sample collections for histopathology and molecular analyses were performed.

Results
Lesions were consistent with systemic infectious disease process targeting pancreas, visceral surfaces, and muscular tunics of the gastrointestinal and respiratory tract. Microscopic findings consistently included necrotizing-histiocytic pancreatitis, visceral celomitis, and leiomyositis, which were the most representative lesions, followed by tracheitis, pneumonia, air sacculitis, nephritis, dermatitis, cellulitis, splenitis, and hepatitis. Histiocytic osteomyelitis with eosinophilic intracytoplasmic inclusion bodies in macrophages were present in two cases. Eosinophilic material morphology, inclusions in the cytoplasm of macrophages, pancreatic acinar cells, and uniformly histiocytic aspect of the inflammatory infiltrates with inclusions in the macrophages together with the almost complete absence of proliferative lesions in the epithelia were not the usual poxviral inclusions or lesions found in the epithelium of infected birds.

Conclusion
All houbaras, irrespective of the origin and year of death, had the same microscopic lesions pattern, suggesting the possibility of a species trend in bustards for this presentation or a specific poxvirus strain tissue tropism. Phylogenetic analysis showed that in all cases strains belonged to Canary Poxvirus-like, clade B.

POSTER ABSTRACTS

Veterinary Pathology: Exotic, wildlife & zoo animals

38 | VISCERAL LEISHMANIASIS IN A BENNETT'S WALLABY (MACROPHUS RUFOGRISEUS)

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Background
Leishmania infantum infection, has been well reported in humans, as well as domestic and wild mammals. *In 2011 it was described for the first time in a Bennett's wallaby (Macropus rufogriseus) kept in a wildlife park in Madrid.* The purpose of this report is to present a new case of fatal visceral leishmaniasis in a captive Bennett's wallaby in Madrid, and to determine the usability of CD1a immunohistochemical staining to detect the amastigotes, as it has been demonstrated in *Leishmania* infection in humans.

Materials & Methods
An adult Bennett's wallaby was found dead in its enclosure at a Zoo in Madrid without significant clinical signs apart of poor body condition. The post-mortem examination was performed in the zoo facilities. Sections from the different organs were collected, fixed in 10% formalin, and processed for hematoxylin and eosin staining. Due to the fact that there was a suspicion of leishmaniasis, based on previously diagnosed cases, PCR for *Leishmania* and CD1a immunohistochemical staining were performed in order to confirm or rule out the etiology.

Results
On gross examination no significant macroscopic lesions where found. Histologically, the spleen parenchyma was almost all replaced by a massive macrophage infiltration containing multiple intracytoplasmic organisms morphologically compatible with *Leishmania* amastigotes. No other significant histological lesions were observed. An intense CD1a immunoreaction was observed. Subsequently PCR confirmed the diagnosis of leishmaniasis.

Conclusion
Our results confirm CD1a staining can be a diagnostic adjunct to confirm leishmaniasis in Wallabies.

POSTER ABSTRACTS

Veterinary Pathology: Exotic, wildlife & zoo animals

50 | A TRICHOEPITHELIOMA IN A 2-YEAR-OLD AFRICAN PYGMY HEDGEHOG

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Background
Spontaneous neoplasms are common in adult African pygmy hedgehogs. Most tumours are malignant, most frequently involving the integument (especially mammary gland), haemolymphatic, digestive and endocrine system.
We report a case of a cutaneous benign trichoepithelioma in an African pygmy hedgehog.

Materials & Methods
A 2-year-old male African pygmy hedgehog was presented with a cutaneous mass on the neck. Regional lymph nodes were unremarkable. The mass was surgically excised. The tissue sample was fixed in 10% buffered formalin and routinely processed for histological examination.

Results
Macroscopically, the mass measured 0,5 centimeter in diameter, had a white to beige colour on cut surface and a moderately firm consistency. Microscopically, a well-circumscribed, non-encapsulated, expansile mass was effacing the dermal and subcutaneous tissue architecture. The mass was composed of variably sized, epithelial islands and cysts showing central, abrupt or gradual, lamellar infundibular or amorphous trichilemmal keratinization, with presence of ghost cells (matrical differentiation). Cysts were variably lined by small basaloid cells resembling matrical cells (reminiscent of hair bulb) and/or squamous epithelium with keratohyalin granules (reminiscent of infundibulum). Large cells with vacuolated, glycogenated cytoplasm were visible (reminiscent of lower isthmic and the inferior portion of the outer root sheath). Local and vascular invasion were absent. The mass appeared completely excised.

Conclusion
This is the first report of a trichoepithelioma in an African pygmy hedgehog. Although common in dogs, hair follicle tumors in hedgehogs are not reported so far. Trichoepitheliomas should be considered as possible differential diagnosis for cutaneous masses in African pygmy hedgehogs.

POSTER ABSTRACTS

Veterinary Pathology: Exotic, wildlife & zoo animals

56 | YERSINIA PSEUDOTUBERCULOSIS INFECTION IN CAPYBARAS FROM A ZOO IN PORTUGAL

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Background
Yersinia pseudotuberculosis is a Gram-negative coccobacillus known to cause pseudotuberculosis, an infection characterized by mesenteric lymphadenitis, acute gastroenteritis and septicaemia. This enteropathogen usually affects rodents, lagomorphs and birds, occasionally infecting domestic animals and humans. Here, we present a fatal case of pseudotuberculosis in a capybara (*Hydrochoerus hydrochaeris*) from a zoo in Portugal.

Materials & Methods
A 6-year-old male capybara was found dead in its zoo enclosure, exhibiting no previous signs of illness. Two females cohabiting the same enclosure were found dead two days later. Tissue samples and smears from the liver, intestines, spleen and lungs of the male specimen were collected for histopathology and bacterial analysis.

Results
Necropsy revealed soft white nodules <5 mm in diameter throughout the spleen, lungs and mesenteric lymph nodes. The nodular lesions described in the lungs and spleen correspond to multiple necrotic cell foci of varying extent, within which it was possible to observe extensive gram-negative bacterial clusters. Also noteworthy in the lungs was the presence of several recently formed thrombi in the small and medium caliber vessels. The use of selective media and biochemical tests confirmed the presence of *Yersinia pseudotuberculosis*.

Conclusion
This is the first report of *Y. pseudotuberculosis* infection in capybaras in Portugal. Investigating the source of this infection is imperative for implementing measures against the spread of this pathogen to other animals and humans, as well as to prevent new cases.

POSTER ABSTRACTS

Veterinary Pathology: Exotic, wildlife & zoo animals

63 | ANALYSIS OF THE CAUSES OF MORTALITY OF WHITE-TAILED EAGLES FROM THE VOJVODINA REGION OVER A FIVE-YEAR PERIOD

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Background
The white-tailed eagle (*Haliaeetus albicilla*) is a strictly protected species in Serbia and the largest part of the population of this species in Serbia inhabits the territory of the Vojvodina province. This study aimed to provide a detailed analysis of the cause of death of this species, with emphasis on the anthropogenic factor.

Materials & Methods
During the five-year period (2018-2023), 27 white-tailed eagles were submitted for forensic pathological examination at the Scientific Veterinary Institute "Novi Sad". All birds were tested for avian influenza and Newcastle virus in accordance with Serbian national surveillance for these diseases. In addition, toxicological analyses were performed in cases of suspected poisoning.

Results
Most of the examined birds were adult, moderately fresh, or advanced decomposed carcasses. Poisoning with the banned pesticide carbofuran was the most common cause of death, detected in 12 cases, while poisoning with the pesticide chlorpyrifos was proven in one case. In three birds, electrocution was found to be the cause of death, followed by extensive thermal damage in feathers and skin and hemopericardium and hemocoeloma. In one case, severe traumatic injuries due to collisions with wind turbines were recorded. The presence of AI and NCD viruses was not detected in any case. Due to pronounced post-mortem changes, the internal organs could not be meaningfully assessed in 10 cases.

Conclusion
This study's results indicate that poisoning with banned pesticides such as carbofuran is still one of the major threats to endangered and protected bird species.

POSTER ABSTRACTS

Veterinary Pathology: Exotic, wildlife & zoo animals

67 | FATAL YERSINIOSIS IN TWO PATAGOINAN MARAS (DOLICHOTIS PATAGONUM) FROM A ZOO: A CASE REPORT

I.C. Šoštarić-Zuckermann[1], B. Artuković[1], Z. Štritof[1], M. Cvetnić[1], J. Bilić[2], I. Bata[2], L. Medven Zagradišnik[1], D. Vlahović[1], I. Ciprić[1], N. Serdar[1], A. Gudan Kurilj[1]
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Background
Yersiniosis is recognized as a prevalent disease within zoo environments, documented across various mammalian species, including the Patagonian maras (*Dolichotis patagonum*). However, comprehensive descriptions of typical lesions in maras remain scarce. Herein, we present pathological findings in a case involving *Yersinia pseudotuberculosis* infection in two Patagonian maras.

Materials & Methods
Within a span of one week, two Patagonian maras from Zagreb Zoo were submitted for necropsy at the Department of Veterinary Pathology, Veterinary Faculty, University of Zagreb. Routine necropsies were conducted, and representative tissue samples were subjected to bacteriological analysis.

Results
Both maras reportedly displayed no clinical manifestations of illness prior to death. Necropsy findings revealed necropurulent foci (abscesses) in the liver, spleen, and lungs of both specimens. Notably, one mara exhibited approximately 1.5 liters of purulent material encapsulated within a cavity formed by the omentum, likely stemming from a ruptured liver abscess. Histopathological examination disclosed multifocal random abscesses with large bacterial colonies. Moreover, in one mara, *Aspergillus* hyphae and fruiting bodies were identified within a larger necrotic focus in the lung. *Yersinia pseudotuberculosis* was isolated from tissues (liver and lungs) sampled from both maras.

Conclusion
This report emphasizes the significance of considering *Y. pseudotuberculosis* as a potential differential diagnosis in cases of acute illness among maras. Furthermore, it provides a detailed description of the characteristic lesions associated with this disease.

POSTER ABSTRACTS

Veterinary Pathology: Exotic, wildlife & zoo animals

73 | HEPATIC B-CELL LYMPHOMA IN A WILD EUROPEAN BADGER (MELES MELES): AN UNUSUAL FINDING DURING TUBERCULOSIS SURVEILLANCE IN THE UNITED KINGDOM

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Background
Hepatic neoplasms are rarely reported in wild European Badgers (*Meles meles*), with a single pelioid hepatocellular carcinoma and four lymphoma cases (two T-cell and two unclassified) found on a literature search.

Materials & Methods
A found-dead elderly male wild Badger underwent postmortem examination for *Mycobacterium bovis* surveillance. The estimated postmortem interval was less than 48 hours. No clinical history was available.

Results
The carcass was icteric and cachexic. There were multifocal white-to-cream firm nodules throughout the hepatic parenchyma (primary site), kidney, and spleen. There was a mild mesenteric lymphadenopathy; all other lymph nodes were macroscopically unremarkable. A population of small neoplastic lymphocytes was identified in the liver, kidney, and spleen. There was a mild parasitic enterocolitis, and mesenteric parasitic lymphadenitis.
On immunohistochemistry, the neoplastic cells were positive for CD79a, CD20, and PAX-5, and negative for CD3. In-silico cross-reactivity via BLASTP supported the positive immunolabelling to a certain degree, with similarity ranging from 57.14% (CD3) to 100% (CD79a). The neoplasia also had a specific but faint Ki67 immunolabelling with an index of 37.4/grid (average in 5 high power fields). Mycobacterial cultures were negative, and no ancillary testing was performed.

Conclusion
Adopting the WHO classification in domestic animals for lymphoid neoplasms, this case was consistent with a primary hepatic, small-cell B-cell lymphoma with a low-to-medium histologic grade.
CD3 and CD79a antibodies have previously been used in badgers. To the best of the authors' knowledge Pax-5, CD20 and Ki-67 have not, and could be used in future research in the European Badger.

POSTER ABSTRACTS

Veterinary Pathology: Exotic, wildlife & zoo animals

77 | GASTRIC CARCINOMA IN A CAPTIVE GREY SEAL (HALICHOERUS GRYPUS): CLINICOPATHOLOGICAL FEATURES

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Background
A 33-year-old female grey seal (*Halichoerus grypus*) was presented with inappetence and progressive weight loss. Medical management included blood analysis (haematology and biochemistry), imaging (ultrasound & X-Rays), and faecal analysis, along with multimodal analgesic support therapy throughout the course of the clinical case. Although the clinical status periodically improved, 6 months after the initial presentation a follow-up blood analysis showed neutrophilic leukocytosis (22.800 × 10^6/L), and the animal showed marked hyporexia, one episode of haemorrhagic vomiting, and increasing general discomfort. Ultrasonographic examination revealed a heterogenous echogenicity structure with anechoic pockets in the cranial abdomen. As the animal was no longer responsive to support therapy and considering her age and overall prognosis, the seal was humanely euthanized on welfare grounds

Materials & Methods
A full postmortem examination was carried out, including complete histopathological evaluation.

Results
The necropsy revealed a marked thickening of the distal oesophagus, cardias, and gastric fundus, which exhibited a locally extensive gastric ulcer, along with multiple nodular lesions involving the omentum. Histopathological examination revealed a metastatic gastric adenocarcinoma involving the oesophagus, diaphragm, small intestine and gastric lymph nodes. An additional microscopic finding included a granulomatous verminous pneumonia.

Conclusion
The literature documenting pinniped neoplasms is expanding gradually, although reports on gastric neoplasia are still rare. To the authors' knowledge, this is the first clinical and pathological report of a gastric carcinoma in a grey seal.

POSTER ABSTRACTS

Veterinary Pathology: Exotic, wildlife & zoo animals

90 | IGNATZSCHINERIA LARVAE INFECTION IN A ROE DEER FAWN (CAPREOLUS CAPREOLUS) WITH MYIASIS

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Background
Ignatzschineria [*Schineria*] *larvae* is a Gram-negative rod that inhabits the intestines of blowflies and maggots like *Wohlfahrtia magnifica*. In animal and human cases of myiasis the bacterium can be transmitted to the host. Though myiasis is more common in animals, reports of *I. larvae* infection mainly exist from humans.

Materials & Methods
In July 2013, a roe deer fawn (*Capreolus capreolus*) was found lethargic next to a road and died soon after being found. The deer was necropsied at the National Veterinary Institute as part of the routine health surveillance of Danish wildlife. Tissue samples were taken for routine histology and bacteriology. After cultivation, bacterial isolates were examined by 16S rRNA gene amplification and sequencing.

Results
The necropsy revealed intracranial bleedings. In the head and neck region, there were several maggot-infestated skin wounds, and hemorrhage in the subcutis, eyes and ears. Areas of lung tissue was consolidated due to multifocal pneumonia. The liver was dark, but otherwise unremarkable. Histology of the lung tissue showed an acute fibrino-necrotizing pneumonia with demarcation of the necrotic tissue. No histologic lesions were present in the liver or brain. From liver and lung, bacterial growth in pure culture was detected and identified by sequencing as *I. larvae*.

Conclusion
This is the first report of a roe deer fawn dying due to bacteremia/sepsis and necrotizing pneumonia caused by myiasis induced infection with *I. larvae*. The fawn had lesions indicative of external force, like a car collision, resulting in blowfly infestation of skin wounds.

POSTER ABSTRACTS

Veterinary Pathology: Exotic, wildlife & zoo animals

91 | CLOSTRIDIAL ENTERITIS OF LORIE PARROTS IN ITALY

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Background
Clostridia are among the most important agents of enteric diseases in poultry. The most common clostridial enteric disease in poultry is necrotic enteritis, caused by Clostridium perfringens, which typically occurs in broiler chickens but has also been diagnosed in various avian species.

Materials & Methods
In January, 10 Lori parrots and 3 intestines were submitted to the Zooprophylactic Institute of Lombardy and Emilia Romagna for pathological examination. All parrots underwent pathologic examination, PCR for: circovirus, polyomavirus, influenza A and avipoxvirus, microbiological and histological examination.

Results
Pathological examination revealed the presence of marked hyperemia in all intestines examined that in some cases were associated with multifocal whitish lesions. I in the livers-lesions ranged from hepatomegaly and diffuse pallor to multifocal whitish lesions of varying width. Three parrots were positive for avian polyomavirus and, from all cloacal swabs *Clostridium perfringens* type A was isolated. Histologically, multiple foci of necrotic-ulcerative enteritis with aggregates of bluish large rod-shaped bacteria were visible. Within the hepatic parenchyma, lesions consisted of areas of necrosis with multifocal bacterial aggregates. Results support the diagnosis of necrotic-ulcerative enteritis

Conclusion
This represents the first case of clostridial enteritis reported in lories in Italy The importation of Lories from multiple geographic areas, the persistence of clostridia in the environment together with the susceptibility of the individuals and transport stress are critical points to consider in the management of these animals for both welfare and biosecurity aspects.

POSTER ABSTRACTS

Veterinary Pathology: Exotic, wildlife & zoo animals

103 | RETROSPECTIVE STUDY OF CAPTIVE RUMINANT NECROPSY CASES FROM A SPANISH ZOO

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Background
In captive settings, animals encounter unique conditions that significantly influence their behaviour and health.

Materials & Methods
This work examined 12 years (2011-2023) of necropsy cases submitted for histopathology examination by a Spanish zoo. One hundred and fifty-nine domestic and nondomestic ruminants belonging to 17 species (Cervidae, Bovidae) were included in this study. Causes of death were classified as: degenerative, developmental,
iatrogenic, idiopathic, infectious, inflammatory, immune-mediated, ischaemic, mechanical, metabolic, neoplastic, nutritional, toxic, trauma, no diagnosis, and euthanasia.

Results
Infectious processes were the main cause of death (21%), being bronchopneumonia and pleuropneumonia most prevalent in impalas and dorcas gazelles. Trauma (12%) affected mainly barbery sheep, impala and dorcas gazelle. Neoplasia was observed in 9% of cases, specially uterine adenocarcinoma. Rumenitis was observed on histopathology in 22% of cases, but this appeared to have little clinical significance. Four out of five amyloidosis cases occurred in dorcas and dama gazelles. For 48% of cases no significant gross or microscopical lesions were observed.

Conclusion
Infectious pneumonia was a common cause of death, particularly in impalas, for which a vaccination regimen was implemented. The incidence of trauma is consistent with other studies and is usually related to intraspecific aggression. Neoplasia was more prevalent in aged animals, particularly in chital and Pere David's deer cases. Amyloidosis has already been reported in some gazelle species. Cause of death analysis in cases without lesions, mostly perinatal, requires further information (clinical, microbiological, behavioural).

POSTER ABSTRACTS

Veterinary Pathology: Exotic, wildlife & zoo animals

121 | CERVICAL PARAGANGLIOMA IN A GUINEA PIG (CAVIA PORCELLUS)

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Background
A 5-year-3-month-old Swiss guinea pig (*Cavia porcellus*), presented with a six-month history of ventral neck swelling, was euthanised due to an unresectable cervical mass.

Materials & Methods
A necropsy was performed, followed by histopathology and immunohistochemical evaluation of the ventral neck mass with antibodies against chromogranin A, thyroglobulin, thyroid transcription factor (TTF)-1, cytokeratin, neuron-specific enolase (NSE), and synaptophysin.

Results
Within the ventral neck an expansile, multi-lobulated mass was identified, which infiltrated into the trachea and oesophagus, and displaced adjacent lymph nodes. The thyroid, however, was not identified. No other mass lesions were identified. Histologically, the mass was composed of polygonal neoplastic cells, which were positively immunolabelled with antibodies against NSE and synaptophysin. Immunolabelling for chromogranin A, thyroglobulin, TTF-1, and cytokeratin was negative.

Conclusion
Based on the anatomical location, histological and immunohistochemical features, the neoplasm is compatible with a cervical paraganglioma. Currently there is no reported case of cervical paraganglioma in the guinea pig.

POSTER ABSTRACTS

Veterinary Pathology: Exotic, wildlife & zoo animals

127 | COINFECTION OF CANINE DISTEMPER AND SARS-CoV-2 IN A FERRET (MUSTELA PUTORIUS FURO), DIAGNOSED BY HISTOPATHOLOGY AND ELECTRON MICROSCOPY

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Background
Ferrets (Mustela putorius furo) are mammals that have gained popularity as pets, so it is important to know the diseases that can affect them.

Materials & Methods
A 4-month-old male ferret was referred to the Hospital of Specialties in Wildlife and Clinical Ethology of the FMVZ-UNAM, with respiratory signs. Due to the clinical condition of the animal, euthanasia was decided. A postmortem study was performed, 1 cm3 samples of all organs were taken and preserved in 10% formaldehyde for H&E staining. Lung and trachea sections were taken for processing by immunohistochemistry, using a polyclonal antibody against the nucleocapsid of the canine distemper virus, as well as 5 mm3 samples of lung and trachea for electron microscopy and PCR.

Results
Histologically, the bronchial and bronchiolar lumens had necrotic debris and shed cells with abundant eosinophilic intracytoplasmic inclusion bodies. Alveolar septa were thickened by lymphocytes, histiocytes, and plasma cells. Immunohistochemistry showed cytoplasmic immunopositivity for distemper nucleocapsid in epithelial cells of the trachea, bronchi, and bronchioles. Electron microscopy of the lung showed abundant fragments of viral nucleocapsids of morbillivirus in the cytoplasm of type 1 pneumocytes. PCR testing was positive for canine distemper virus and SARS-CoV-2.

Conclusion
In this study, clinical history, postmortem examination, and diagnostic tests were vital to confirm the etiological agents involved in interstitial pneumonia, also supporting the coinfection with SARS-CoV-2.

POSTER ABSTRACTS

Veterinary Pathology: Exotic, wildlife & zoo animals

136 | DISTRIBUTION OF PORCINE CIRCOVIRUS 2 IN WILD BOAR IN ESTONIA

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Background
Porcine Circovirus 2 (PCV2) causes post-weaning multisystemic wasting syndrome and PCV2-related diseases. As according to literature wild boars are among spreaders of virus, aim of the study was to detect the spread of PCV2 in pigs in Estonia, detecting distribution areas of PCV2 in wild boars and comparing these with existing distribution areas of PCV2 in domestic pigs.

Materials & Methods
Samples of 1-4 years old wild boars were selected from all Estonian counties. A total of 460 blood samples were examined with ELISA method (Ingezim Circo IgG) and randomly selected tissue sections from wild boar mesenteric lymph nodes by histopathological and immunohistochemical (IHC) study.
The organ samples were tested for PCV2 by RT-PCR and immunohistochemical stainings. For IHC formalin-fixed, paraffin-embedded tissue sections were deparaffinised with xylene, rehydrated in alcohol, endogenous peroxidase activity was blocked with 0,6% hydrogen peroxide. PCV2 monoclonal antibody (by Danish Institute for Food and
Veterinary Research) 1:300 was used as primary antibody and EnVision+™ System (DakoCytomation) for incubation.

Results
Studies showed widespread distribution of PCV2 in wild boars in Estonia as ELISA test detected PCV2 antibodies in 92% of samples.
Histopathological study revealed depletion of lymphocytes and proliferation of histiocytes. Locations of the most infected wild boars overlapped with locations of 96% of infected domestic pig farms.

Conclusion
Study revealed that domestic pig infestation is higher in areas of higher wild boar infestation.
As the antibodies are present in 92% of animals exposed to PCV2, probability that wild boars are spreading the virus to domestic pigs is high.

POSTER ABSTRACTS

Veterinary Pathology: Exotic, wildlife & zoo animals

156 | BLUNT FORCE TRAUMA IN THE CANARIAN HOUBARA BUSTARD (CHLAMYD-OTIS UNDULATA FUERTAVENTURAE) PRODUCED BY COLLISION WITH OVER-HEAD LINES

C.M. Suárez-Santana[1], L. Marrero-Ponce [1], J. Navarro-Sarmiento [1], C. Rivero Herrera [1], R. Grandía Guzmán [1], A. Colom-Rivero[1], Ó. Quesada-Canales[1], E. Sierra[1], A. Fernández[1]
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Background
The mortality of birds resulting from collisions and electrocutions with overhead lines, such as power lines, and phone line among others, has been implicated in the decline of various avian species globally. Specifically, overhead line collisions pose a significant threat to the conservation of the Canarian houbara bustard (Chlamydotis undulata Fuertaventurae), an endangered sub-species endemic to the Canary Islands.

Materials & Methods
This study centres on the postmortem findings of Canarian houbara bustards that have collided with overhead lines, providing insights into the post-collision outcomes for these birds.

Results
A complete standardized necropsy of nine Canarian houbara bustards revealed that polytrauma was the cause of death in all cases. The most notable gross lesions associated with trauma included bone fractures, soft tissue lacerations, haemorrhages, luxations, and hemocoelom, with certain body regions being more frequently affected. Histopathology, immunohistochemistry, and en-tomology analysis confirmed that numerous birds survived the initial trauma, exhibiting varia-ble survival intervals before succumbing to their injuries.

Conclusion
We concluded that when a houbara bustard collide with an overhead line it frequently survives the initial trauma. The histopathology, immunohistochemistry, or entomologic analysis may be helpful to approximate the timing interval between trauma and death.

POSTER ABSTRACTS

Veterinary Pathology: Exotic, wildlife & zoo animals

157 | PATHOLOGICAL STUDY OF A TRAUMATIC ANTHROPOGENIC INJURY IN THE SKELETON OF A SPINY BUTTERFLY RAY (GYMNURA ALTAVELA)

G. Montero-Hernández[1], A. Castro-Alonso[1], M.J. Caballero[1], Á. Curros-Moreno[2,3], C.M. Suárez-Santana[1], M.A. Rivero[1], L. Caballero-Hernández[1], M. Encinoso[4], A. Fernández[1]
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Background
External injuries in elasmobranchs are frequent findings, either due to natural interactions or anthropogenic activities. However, there is a general lack about the pathological description of severe traumatic lesions.
This work provides an overview of the clinical presentation, diagnostic imaging and pathological features of a traumatic skeletal injury in a spiny butterfly ray (*Gymnura altavela*).

Materials & Methods
An adult female was found lethargic in the bottom of the coast of Gran Canaria, with an external circular incised-contused traumatic lesion of 2 cm diameter in the scapulocoracoid cartilage. It was captured and transferred to the Poema del Mar Aquarium facilities for its clinical evaluation and treatment options. Despite these efforts, the animal eventually died and was transfer to the IUSA for its routinely pathological diagnosis, including a Computed Tomography (CT) study.

Results
A notable reduction on the haematocrit and the hepatosomatic index confirmed a chronic debilitation process. The CT study revealed a comminuted fracture on the right scapulocoracoid cartilage, impacting the articular surfaces of the pectoral arch. The main pathological findings showed the disorganization of the tesserae layer, macroscopically appearing as whitish square to rectangular geometric pieces. Histologically, these pieces of tesserae were separated from the unmineralized cartilage core and ripped out from the perichondrium. In the adjacent tissues, an intense infiltrate of granulocytes and fibrous connective tissue, oedema and haemorrhages were observed.

Conclusion
This study reports the first comprehensive description of skeleton trauma in a spiny butterfly ray, including the clinical presentation, diagnostic imaging and the anatomopathological features.

POSTER ABSTRACTS

Veterinary Pathology: Exotic, wildlife & zoo animals

163 | DEADLY SERRATIA MARCESCENS INFECTION IN A BOTTLENOSE DOLPHIN (TURSIOPS TRUNCATUS)

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Background

Bacterial septicemia is well described in dolphins and numerous bacteria genera, such as *Erysipelothrix, Streptococcus, Aeromonas, Pseudomonas*, etc., have been associated with it. *Serratia marcescens* is a gram-negative opportunistic pathogen, scarcely described in veterinary literature, which has been referred as an etiological agent of nosocomial infections in humans, affecting the respiratory and urinary systems. This case report aims to describe the findings in a bottlenose dolphin (*Tursiops truncatus*), under human care, with a septicemia due to *Serratia marcescens*.

Materials & Methods

A 21-year-old, male, bottlenose dolphin with clinical history of anorexia and renal azotemia was brought for a full postmortem study. Histopathology was performed and samples from blood, urine, liver and kidney were submitted for microbiology culture.

Results

On gross examination, the lungs presented multiple coalescent greyish-white nodules, and both kidneys showed multiple lobes with areas of hemorrhage. The right ureter was distended and congested. Histopathology revealed a severe necrosuppurative pyelonephritis with Gram-negative bacteria and bacterial emboli; a broncho-interstitial pneumonia with embolic foci was observed as well. In aerobic and anaerobic cultures, *Serratia marcescens* was isolated from blood, urine, kidney and lung. Other findings included a very chronic and depressed scar in the right pectoral fin, which precluded the dolphin from moving it properly. There were also tattoo like lesions around the rostrum.

Conclusion

The pathological findings together with the isolation of *Serratia marcescens* in various tissues, including blood, are consistent with a bacterial sepsis. An ascending urinary infection is presumed to be the source of infection.

POSTER ABSTRACTS

Veterinary Pathology: Exotic, wildlife & zoo animals

171 | CHARACTERIZATION OF AMYLOIDOSIS IN EURASIAN STONE-CURLEWS (BURHINUS OEDICNEMUS) FROM THE CANARY ISLANDS

L. Marrero-Ponce[1], A. Fernández[1], C. Rivero-Herrera[1], J. Navarro-Sarmiento[1], R. Grandía Guzmán[1], C. Manuel Suárez-Santana[1], Ó. Quesada-Canales[1]
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Background
Amyloid deposits consist of fibrils' accumulation resulting from different precursor proteins. Their identification is crucial for adequate disease prognosis and treatment. In avian amyloidosis, most reports do not determine which fibrils are implicated or apply out-of-date methods. Other studies use antibody-based methods, but their use has important drawbacks, especially in scarcely studied species. Currently, laser microdissection followed by tandem mass spectrometry (LMD-MS/MS) is considered the gold standard for amyloid characterization.

Materials & Methods
30 specimens of Eurasian stone-curlews (*Burhinus oedicnemus*) were necropsied. Tissue samples were taken, routinely processed, and stained with HE. When lesions consistent with amyloid deposit were observed, Congo red stain and polarized light were employed. After confirmation of amyloidosis in 7 individuals, selected formalin-fixed paraffin embedded samples of 5 animals were processed and subjected to LMD-MS/MS.

Results
7 Eurasian stone-curlews showed macroscopic and histologic lesions consisting of amyloidosis, including hepatomegaly, splenomegaly, nephromegaly and the presence of extracellular amorphous hyaline deposits with a red discoloration when stained with Congo red and yellow-red-green birefringence under polarized light. Serum amyloid A (SAA) was the amyloid precursor protein most detected in all animals analyzed but one, in which apolipoprotein A-I (ApoAI) was ampler. ApoAI and apolipoprotein A-IV (ApoAIV) were present in all samples. Heparan sulphate proteoglycan 2 (HSPG2) was present in four animals.

Conclusion
The amyloid proteome of the 5 specimens analyzed consisted of SAA, ApoAI and ApoAIV and, frequently, HSPG2. ApoAI surpassed SAA in one case, consequently this lipoprotein should be considered as potential precursor protein in avian species.

POSTER ABSTRACTS

Veterinary Pathology: Exotic, wildlife & zoo animals

177 | HISTOPATHOLOGICAL CHANGES IN HUNTER-KILLED WILD BOARS NATURALLY INFECTED WITH AFRICAN SWINE FEVER VIRUS GENOTYPE II

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Background
Numerous studies have been published about lesions caused by African swine fever (ASF) virus Genotype II in naturally infected domestic pigs and experimentally infected animals. However, there is paucity of data regarding lesions caused by ASF virus in wild boars during the current ASF panzootic in Europe. The aim of this study was to determine histopathological changes in hunter-killed wild boars that are PCR-positive for ASF.

Materials & Methods
Tissues were selected from wild boars (n=6) that were killed during active ASF surveillance in Latvia (2018-2023) and were PCR-positive for ASF virus genome. HE-stained tissue sections of lymphoid organs (spleen, lymph node, tonsil) and kidneys were examined and scored according to a system adapted from the previously published protocol.

Results
Mild to moderately severe lesions characteristic of ASF were found in all animals. These included vascular thrombosis, tubular necrosis, hemorrhage and lymphoplasmacytic interstitial inflammation in the kidneys and lymphoid depletion in the lymphoid organs. Concurrently, lymphoid follicular hyperplasia was observed in lymph nodes and tonsils and extramedullary hematopoiesis was observed in the spleen. In 2/6 animals infiltration of multinucleated giant cells was found in the lymph nodes possibly indicating concurrent infection with porcine circovirus type 2.

Conclusion
All six hunter-killed wild boars positive for ASF virus genome had mild to moderate lesions characteristic to ASF in the kidneys and lymphoid organs. These lesions may represent an early clinical or subclinical stage of natural ASF infection. In some animals additional background lesions were detected suggestive of infection with other pathogens.

POSTER ABSTRACTS

Veterinary Pathology: Exotic, wildlife & zoo animals

195 | FATAL NECROTIC-HAEMORRHAGIC PANCREATITIS ASSOCIATED WITH VIBRIO HARVEYI INFECTION IN A SPINY BUTTERFLY RAY (GYMNURA ALTAVELA)

G. Montero-Hernández[1], A. Castro-Alonso[1], M.J. Caballero[1], Á. Curros-Moreno[2,3], D. Padilla[1], L. Caballero-Hernández[1], E. Montesdeoca-Morales[1], A. Fernández[1]
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Background
Pancreatitis is a common mild finding in dead elasmobranchs, mostly attributed to parasite migration. However, it is rarely associated with severe clinical symptoms or as a primary cause of death. To date, no bacterial agents have been clearly associated to fatal pancreatitis in elasmobranchs.

Materials & Methods
An adult male spiny butterfly ray (*Gymnura altavela*), housed in a private aquarium under human care, showed non-specific signs of weakness. Despite treatment, the animal's condition deteriorated rapidly and, following its death, it was referred to IUSA to perform pathological investigation. Samples were taken for histological study and bacterial culture.

Results
The main macroscopic finding was a focal (2-3 cm) greenish-white necrotic area in the pancreas. Cutting this lesion revealed a purulent exudate. In addition, scattered white foci were observed throughout the pancreatic tissue. Microscopically, extensive areas of pancreatic necrosis with severe inflammatory reaction and haemorrhage were observed. Moreover, multifocal infiltrations of inflammatory cells (granulocytes and macrophages) were present in the interacinar spaces. *Vibrio harveyi* was isolated from a sample of the pancreas.

Conclusion
This work describes the histopathological findings of a severe necrotic-haemorrhagic pancreatitis and its association with *Vibrio harveyi* infection as potential cause of death in a spiny butterfly ray.

POSTER ABSTRACTS

Veterinary Pathology: Exotic, wildlife & zoo animals

200 | PLASTIC CONTAMINATION AND LESIONS OBSERVED IN CORY'S SHEARWATERS (C. BOREALIS) FROM THE AZORES ARCHIPELAGO - A PILOT STUDY

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Background
Growing evidence shows how several anthropogenic stressors, such as light and plastic pollution affect marine birds. Procellariiformes seabirds in particular are especially susceptible to plastic contamination, ingesting plastic debris, and feeding it to their young. The aim of this study was to assess the lesions and plastic content in the digestive system of Cory's shearwaters (*Calonectris borealis*) found dead in the Azores archipelago during SOS Cagarro campaign (2023).

Materials & Methods
Data regarding the necropsy of 64 Cory's shearwaters was collected. Organ samples from the specimens that displayed fewer autolytic changes were fixed in 10%buffered formalin and processed for routine paraffin embedding and histological evaluation (n=9).

Results
The majority of the animals (39/60.93%) presented good body condition. A high prevalence (52/81.25%) of plastics ingestion was observed both in chicks and adults, with items recovered mainly from the gizzard. Histopathology revealed the presence of non-organic material with features consistent with microplastics (visible under polarized light and fluorescent) along the digestive tube. Multifocal lymphocytic infiltrate was observed in the liver of 7 animals (77.7%), followed by sub capsular renal haemorrhage and chronic esophagitis each displayed by 3 animals (33.3%).

Conclusion
Plastic ingestion by *C. borealis* appears to be a common trait in chicks and adults. Chronic hepatitis was present in almost all the animals sampled for histopathology. Regular monitoring of the health status of these bird species is pivotal to the understanding of the detrimental effects of plastics in marine birds and to the extent and severity of marine plastic contamination in the North Atlantic.

POSTER ABSTRACTS

Veterinary Pathology: Exotic, wildlife & zoo animals

202 | HEALED BROKEN HEART - ILLEGAL HUNTING OF A BARBARY FALCON (FALCO PEREGRINUS PELEGRINOIDES)

L. Marrero-Ponce[1], A. Fernández[1], C. Rivero-Herrera[1], J. Navarro-Sarmiento[1], R. Grandía Guzmán[1], C. Manuel Suárez-Santana[1], Ó. Quesada-Canales[1]
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Background
Hunting of raptors is forbidden in many countries, Spain included. Despite this prohibition, illegal shooting is currently considered an important conservation problem for this kind of avian species.

Materials & Methods
One adult specimen of Barbary falcon (*Falco peregrinus pelegrinoides*), an endangered species, was subjected to a complete standardized necropsy. Tissue samples were fixed in 4% buffered formalin, routinely processed, and stained with HE. Masson's trichrome stain of heart tissue was also performed.

Results
Complete subcutaneous and visceral fat depletion and severe pectoral muscle atrophy were observed. Comminute closed fractures were present in both tibiotarsus bones. On the right leg, two lead pellet were present in addition to a focally extensive subacute bruise. On the left leg, a deformed lead pellet was extracted adjacent to an area of dense connective tissue that surrounded the fracture. One last pellet was lodged in the visceral surface of the right ventricle wall. It was surrounded by a thin layer of connective tissue and few epithelioid macrophages. Scar tissue was seen in the parietal side of the right ventricle. By Masson's trichrome stain a path of fibrous tissue from the parietal plane to the visceral one, passing through the interventricular septum, was demonstrated. No lesions were observed in other tissues.

Conclusion
The bird survived afterward a shooting event, which did not lead directly to death. Animal capability of movement and hunting was impaired due to gunshot lesions, leading to a probable death by inanition.

POSTER ABSTRACTS

Veterinary Pathology: Exotic, wildlife & zoo animals

208 | OVARIAN DYSGERMINOMA IN A 14-YEAR-OLD ROCK WALLABY (PETROGALE XANTHOPUS)

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Background
A geriatric female Rock wallaby (*Petrogale xanthopus*) with severe, progressive clinical signs of weakness and oral disease which did not resolve after dental extraction and treatment with anti-inflammatory drugs and antibiotics was humanely euthanized at a zoological facility. The animal had a history of multiple births.

Materials & Methods
Necropsy was performed and tissues from every organ system were processed for routine histopathology at the Zoo and Wildlife Pathology Service of the Complutense Veterinary Teaching Hospital of Madrid.

Results
The gross examination revealed dental loss and gingival necrosis. The ovaries were obliterated by large, white, firm masses that compressed the scant, remaining parenchyma. Similar masses were found multifocally within the liver, kidneys, and mesentery, and hemoperitoneum. Histopathology of the neoplasms revealed an infiltrative mass, with polygonal cells, arranged in cords and packets, fine fibrovascular stroma, marked anisocytoses and numerous mitotic figures, consistent with a dysgerminoma. Additionally, the uterus had a severe, chronic, cystic endometrial hyperplasia. The remaining lesions were associated with senility and chronic disease.

Conclusion
Ovarian tumours have not been reported in macropods which makes this case unique. Hyperoestrogenism is commonly associated with dysgerminomas and was likely the cause of the cystic endometrial hyperplasia. This finding provides new insights for a better understanding of the pathology of the reproductive tract in macropods.

POSTER ABSTRACTS

Veterinary Pathology: Exotic, wildlife & zoo animals

218 | AORTIC ANEURYSM AND RENAL DISEASE IN A 3-YEAR-OLD RABBIT WITH RABBIT HEMORRHAGIC DISEASE

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Background
The necropsy of a 3-year-old rabbit with lethargy and acute death was performed. Its companion died with the same symptoms 24 hours earlier, and at that time, a blood test consisting of hemogram and complete biochemistry was performed on the necropsied rabbit, which showed no abnormalities. Subsequently, the rabbit exhibited severe lethargy and died in the following hours. Both animals lived in a private garden and had no history of vaccination. Their diet consisted of fresh grass, mixed grass hay and pellets, all provided ad libitum.

Materials & Methods
Complete necropsy was performed and tissues from multiple organ systems were processed for histopathology at the Pathology Service, Complutense Veterinary Teaching Hospital of Madrid, Spain, following routine laboratory procedures.

Results
Histopathology revealed a severe systemic vascular disorder with hemorrhages and congestion in the serosal membranes, adrenal glands, kidneys, digestive tract, trachea, and lungs. Additionally, there was a severe suppurative necrotizing hepatitis. The aortic artery contained a saccular aneurysm with mural calcification. The kidneys contained membranous glomerulonephritis and glomerulosclerosis with nephrocalcinosis. The parafollicular cells of the thyroid were hyperplastic.

Conclusion
The gross and histopathological exam in this 3-year-old rabbit revealed lesions compatible with rabbit hemorrhagic disease, as well as renal degeneration with glomerulosclerosis, and a severe aneurysm in the aortic artery with calcification not related with the hemorrhagic disease. The parafollicular hyperplasia indicated alterations in the calcium metabolism. Renal disease and its consequences in middle-aged rabbits should be considered, even in the absence of biochemical alterations in parameters related to renal function.

POSTER ABSTRACTS

Veterinary Pathology: Exotic, wildlife & zoo animals

219 | OUTBREAK OF MORTALITY ASSOCIATED TO AVIAN POX INFECTION IN FREE-RANGING HOUSE SPARROWS (PASSER DOMESTICUS)

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Background
Avian pox is a slow-spreading viral disease that occurs in wet or dry form, caused by viruses from the family *Poxviridae*. Cutaneous/dry form typically affects the unfeathered areas and is not generally considered the direct cause of mortality but may increase the risk of trauma and secondary infections.

Materials & Methods
Six (3/6 male and 3/6 female) adult house sparrows were necropsied, from December 2023 to February 2024, in Barcelona Zoo. Four were found dead and two severely weak and euthanised.

Results
Grossly, all the animals showed multifocal small scab-like nodules predominantly within feather follicles. Lesions were also located around the beak and eyes. Animals showed a poor body condition (3/6), trauma (2/6) and delayed pneumatization of the skull (2/6). Microscopically, multifocal proliferative and crusting dermatitis with pterylitis or folliculitis (6/6) and nasal cavity involvement (3/6) was seen with intracytoplasmic Bollinger bodies in keratinocytes. Viral inclusions were also observed in bronchial epithelium (2/6).
Additional microscopic findings were septicaemia (2/6), mycotic pneumonia (1/6), granulomatous splenitis (1/6), hepatic necrosis (1/6), lymphoid depletion (1/6), intestinal parasites (2/6) and renal trematodes (1/6).
The definitive diagnosis was cutaneous avian pox with secondary fatal infections and delaying of bone development. The ultimate cause of death for two birds was trauma and for one was severe mycotic pneumonia.

Conclusion
The presence of skin lesions localized in feather follicles must include cutaneous avian pox as a differential diagnosis, despite not being the classical location. Delayed skull pneumatization can have multiple causes, including viral infections, although further studies are needed.

POSTER ABSTRACTS

Veterinary Pathology: Exotic, wildlife & zoo animals

244 | LOCAL LUNG/LYMPH NODE IMMUNE RESPONSE TO MYCOBACTERIUM BOVIS CHALLENGE AFTER M. BOVIS HEAT-INACTIVATED VACCINATION IN EUROPEAN BADGER USING BAITS

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Background

In some regions of Europe, the badger (*Meles meles*) is recognised as a tuberculosis (TB) reservoir host involved in the transmission of the disease to cattle; thus, vaccination is being considered as a way of controlling the disease. The study of granulomas by immunohistochemistry (IHC) is a tool for understanding the mechanisms of vaccine protection.

Materials & Methods

The objective of this study was to determine the proportions of cells (macrophages, T and B lymphocytes and plasma cells) and proteins/cytokines (TGF-β, IL-10 and FoxP3) present in TB granulomas of the right middle lung lobe and right bronchial lymph node in control (n=7), and vaccinated badgers (n=8) with a bait containing a heat-inactivated strain of *Mycobacterium bovis* (HIMB), experimentally infected with *M. bovis* via the endobronchial route targeting the right middle lung lobe.

Results

Macrophages were the most abundant cells in granulomas, followed by plasma cells and B and T lymphocytes in both groups. The cell patterns observed did not show any statistical difference in local immune response between the control and vaccinated groups or in the tissues. However, two vaccinated badgers (called divergent responders, which showed the highest lesion and bacterial load scores) seemed to react differently from all other badgers (called vaccinated and standard responders), e.g., they showed significantly higher FoxP3 in lymph nodes.

Conclusion

The results suggest a potential T cell exhaustion and tolerization in divergent badgers. In the remaining badgers a non-specific innate immune response was observed.

POSTER ABSTRACTS

Veterinary Pathology: Exotic, wildlife & zoo animals

246 | TOXOPLASMOSIS IN NON-HUMAN PRIMATES: A CASE REPORT IN AN OLD-WORLD MONKEY (CYMONOLGUS MACAQUE).

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Background
Toxoplasmosis, caused by the intracellular protozoan parasite *Toxoplasma gondii*, is a significant zoonotic disease affecting various vertebrate hosts. New world non-human primates are highly susceptible to *T. gondii*. However, old world primates, including macaques, exhibit marked resistance to the infection. There is sparce knowledge of toxoplasmosis in old-world non-human primates due to the scarcity of cases. The aim of this study is to provide a detailed analysis of a cynomolgus macaque (*Macaca fascicularis*) diagnosed with toxoplasmosis.

Materials & Methods
A 3-year-old male cynomolgus macaque exhibited notable clinical symptoms including progressive weight loss, respiratory distress, and fever. Macroscopic examination revealed no abnormalities. Tissue samples underwent HE staining, immunohistochemistry and PCR against *T. gondii.*

Results
Microscopic evaluation showed severe interstitial pneumonia characterized by necrosis, edema, and hyperplasia of type II pneumocytes. Small intestine exhibited enteritis primarily affecting the submucosa. Both lesions exhibited intracytoplasmic protozoan structures, occasionally found extracellularly. All other organs were not affected. Immunohistochemistry demonstrated strong positivity against *T. gondii*, mainly in the cytoplasm of intraalveolar and interstitial macrophages, epithelial cells of the intestinal mucosa, and endothelial cells. PCR confirmed the presence of *T. gondii* in both lungs and intestine.

Conclusion
In contrast to new world primates, reports of toxoplasmosis in old world species are very scarce. This case highlights the potential for clinical and histological manifestations even in presumed resistant species. Thereby emphasizing the need for vigilance and contributing to the understanding of toxoplasmosis in old world non-human primates.

POSTER ABSTRACTS

Veterinary Pathology: Exotic, wildlife & zoo animals

250 | OVARIAN TERATOCARCINOMA WITH INTRA-ABDOMINAL DISSEMINATION IN A CYNOMOLGUS MACAQUE (MACACA FASCICULARIS)

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Background
Ovarian teratocarcinomas are malignant tumours consisting of undifferentiated tissues along with mature elements from two or three germ layers. These tumours are rare and have been reported in dogs, mice, birds, and horses. Here, we present a rare case of ovarian teratocarcinoma in a cynomolgus macaque.

Materials & Methods
An adult female macaque was brought to the Faculty of Veterinary Science, Mahidol University, Thailand, due to severe abdominal enlargement. Euthanasia was performed, and the carcass was submitted for further histopathological and immunohistochemical evaluation.

Results
The necropsy examination revealed intra-abdominal masses, including an 8 x 8 x 5 cm multilobulated ovarian mass, an 18 x 15 x 10 cm mass containing viscous fluid, and a 15 x 13 x 7 cm mass with an irregular surface. There was no evidence of lymph node enlargement or distant metastasis. Histologically, representative sections of all masses showed well-differentiated tissues from the three embryonic germ layers: ectoderm, containing squamous epithelium, sebaceous gland, and nerve tissue; mesoderm, containing cartilage and bone; and endoderm, consisting of ciliated columnar epithelium with goblet cells. Immature tissues, including undifferentiated spindle cells, and poorly differentiated glandular tissues were also observed. The ectodermal and mesodermal tissues were immunoreactive for cytokeratin AE1/AE3, desmin, and vimentin respectively.

Conclusion
Teratocarcinomas are uncommon tumours composed of undifferentiated or embryonal tissues. They typically grow rapidly and have a high potential for dissemination and metastasis. This report presents a rare case of ovarian teratocarcinoma in a macaque, as documented through pathological and immunohistochemical evaluation.

POSTER ABSTRACTS

Veterinary Pathology: Exotic, wildlife & zoo animals

269 | RETROSPECTIVE CHARACTERIZATION OF VIRAL INDUCED SKIN AND GENITAL PROLIFERATIVE LESIONS IN STRANDED CETACEANS OFF THE COASTS OF SPAIN

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Background
Proliferative skin lesions are a common finding in free-ranging cetaceans all over the world. Poxvirus and herpesvirus have been associated with these lesions in skin and genitals. However, a causal-effect relationship between the lesion and the virus is often obscure. This study was designed to review the histopathological features of skin and mucocutaneous proliferations in cetaceans stranded on the Spanish coasts of Valencia and Cantabria between the years 2019 and 2021, and their association with herpesvirus and poxvirus.

Materials & Methods
Skin and/or genital samples were selected from 7 striped dolphins (*Stenella coeruleoalba*) and 1 bottlenose dolphin (*Tursiops truncatus*) from the archives of the Zoo and Wildlife Pathology Service of the UCM Veterinary Teaching Hospital. Skin (n=6) and genitalia (2 penes/1 vagina) were examined by routine histopathology and poxvirus and herpesvirus were analysed by PCR.

Results
The 6 skin samples had chronic proliferative dermatitis, 4 of which had cytoplasmic inclusions compatible with poxvirus. Cetacean poxvirus-1 was identified in 3 of those four. Four out of the six were positive for alpha-herpesvirus. Vaginal and penile samples had lymphoplasmacytic inflammation without visible inclusion bodies and were positive for gamma-herpesvirus.

Conclusion
Proliferative dermatitis with poxviral inclusions was usually associated with the molecular detection of cetacean poxvirus-1, suggesting a causal-effect relation between lesion and virus. Alpha-herpesvirus coinfection in one animal and the detection in another animal with poxviral-like inclusions yet negative for poxvirus, raises questions on the role alpha-herpesvirus plays and the possible interaction between both viruses. Gamma-herpesvirus was only detected in genitalia.

POSTER ABSTRACTS

Veterinary Pathology: Exotic, wildlife & zoo animals

270 | OUTBREAK OF AVIAN POX IN AVIARY BRED SAKER FALCONS (FALCO CHERRUG)

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Background
Avian pox is a worldwide-distributed disease, with cases described in more than 300 species of birds due to a virus of the genus Avipoxvirus and the family Poxviridae. The main transmission mechanisms are through blood-sucking arthropods and direct contact. The disease manifests predominantly in two forms, cutaneous (dry) and diphtheroid (wet). In falcons mainly dermatological problems occur, depending on their localization, with complications.

Materials & Methods
Clinical examination was performed of 33 aviary-bred Saker falcons (*Falco cherrug*), part of a reintroduction program conducted by the NGO,,Green Balkans". Histopathological examination was done on macroscopically identified nodules, biochemical analysis on blood samples.

Results
Eleven Saker falcons exhibited prominent nodules, hyperkeratotic, and ulcerative lesions, primarily on the digits, corners of the mouth, eyelids, and around the nostrils. Some birds had extensively grown lesions on the digits, resulting in partial amputation. Histopathological examination confirmed hyperplasia and swelling of the stratum spinosum, with eosinophilic intracytoplasmic inclusions (Bollinger bodies). Biochemical analysis revealed elevated blood urea nitrogen levels (reference values were obtained from the same birds).

Conclusion
In this outbreak the birds recovered without any remaining rough cicatrixes and they exhibited normal breeding behaviour, and great hatching success during the next breeding season. In wild birds, the disease can cause complications such as difficulty hunting and feeding, due to the digit loss, and secondary bacterial infections as a result of impaired skin barrier. This would adversely affect the population of this globally threatened species.

POSTER ABSTRACTS

Veterinary Pathology: Exotic, wildlife & zoo animals

276 | DEPIGMENTATION MACULES IN A HARBOUR SEAL (PHOCA VITULINA): VITILIGO OR PIEBALDISM?

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Background
Zoomarine's pinniped collection is under a preventative medicine programme, based on voluntary medical behaviours, to ensure their welfare and health status.
A 6-year-old male *Phoca vitulina* housed at Zoomarine since 2019, showed randomly dispersed areas of skin discoloration, consistent with piebaldism or vitiligo. These lesions have remained relatively stable throughout the last years. This individual showed an unremarkable medical history, and has never manifested dermatological problems, except for occasional solar erythema in the areas of depigmentation, associated with high sun exposure.

Materials & Methods
This animal shows variably sized dispersed depigmented macules, mostly on the torso and on the left flipper. Skin biopsy was obtained from one of the macules. Routine histopathology and immunohistochemistry with Melan A (DAKO, clone A103) were performed.

Results
The biopsy revealed a complete absence of melanin-containing cells in the epidermis or dermis in an otherwise normal skin. However, Melan A immunohistochemistry regularly displayed melanocytes in the basal layer of the epidermis.

Conclusion
While piebaldism has been documented in pinnipeds, probably based solely on visual examination, the present case does not align entirely with this diagnosis. Piebaldism is defined as a lack of melanin producing cells from birth. The observed growth in some macules and the presence of nonfunctional epidermal melanocytes suggest a diagnosis of vitiligo, a progressive autoimmune condition.
This case highlights the importance of histopathological examination in distinguishing between piebaldism and vitiligo in pinnipeds. Further research into vitiligo among pinnipeds could provide valuable insights into the pathogenesis and management of this condition.

POSTER ABSTRACTS

Veterinary Pathology: Exotic, wildlife & zoo animals

281 | IMMUNOHISTOCHEMICAL CHARACTERISTICS OF TESTICULAR TUMORS IN TWO SPECIES OF ZOO FELIDS

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Background
Testicular neoplasms are rarely observed in both domestic and wild felids, although cases of seminoma, Sertoli cell tumors, and interstitial (Leydig) cell tumors (LCT) have been reported. Immunohistochemisty (IHC) is essential not only in cases where the tumor origin is ambiguous but also when a typical histological tumor pattern is evident in a non-conventional species. This study presents a panel of IHC markers in testicular tumor of the Siberian tiger (*Panthera tigris altaica*) and Eurasian lynx (Lynx lynx).

Materials & Methods
Histopathology and IHC were conducted on two testicular tumors collected during routine necropsies of a Siberian tiger and a Eurasian lynx, both delivered from the Osijek Zoo to the Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Zagreb. Semi-quantitative IHC analyses were performed using vimentin, Melan A, desmin, cytokeratin, c-Kit and Ki-67.

Results
In the tiger, histological analysis identified the testicular tumor as LCT, whereas in the lynx, it exhibited characteristics of a rete testis adenoma (RTA). In both tumors, vimentin showed strong positivity, while desmin and c-Kit were negative. LCT displayed strong Melan A positivity, and in RTA, Melan A and cytokeratin mild positivity in individual cells. LCT and RTA showed low Ki-67 labeling index of 3% and 1.7%, respectively.

Conclusion
In conclusion, LCT and RTA, exhibited immunoreactivity to the same IHC markers as observed in same tumors found in other species in which these tumors more commonly occur, with similar benign biological behavior.

POSTER ABSTRACTS

Veterinary Pathology: Exotic, wildlife & zoo animals

286 | GRANULOMATOUS ENTERITIS DUE TO MYCOBACTERIUM AVIUM SUBSP AVIUM IN AN ALPACA

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Background
Non-tuberculous mycobacteria (NTM) comprise the *Mycobacterium avium* complex, including *Mycobacterium avium* subsp. *paratuberculosis* (MAP) and *Mycobacterium avium* subsp. *avium* (Maa).
MAP is a causative agent of granulomatous enteritis (Johne's disease), mainly involving ruminants, whereas Maa is the main tuberculosis pathogen of birds, that can occasionally infect mammals.
This work describes a case of a granulomatous enteritis due to Maa in a young alpaca (*Lama pacos*) in Ireland.

Materials & Methods
An eight-month-old alpaca with a two-week history of diarrhoea was submitted for a necropsy. Selected tissues were submitted for histology and mycobacterial culture (*Mycobacterium bovis* and MAP). Ziehl-Neelsen (ZN) stain was performed on smears from cultures displaying growth, and PCR speciation was conducted on those positive for acid-fast bacilli.

Results
Necropsy revealed severe emaciation, mesenteric lymphadenomegaly, loose intestinal contents, and multifocal tan pinpoint to larger spherical lesions (2mm-5mm) throughout liver and spleen. Histologically, there was a marked granulomatous enteritis with moderate villus atrophy, lymphangiectasia, and severe diffuse necrotizing granulomatous lymphadenitis with lymphangitis; thrombosis and multifocal to coalescing moderate to severe granulomas within the spleen and liver were also present. Large numbers of ZN positive bacilli were seen within the tissues. NTMs were largely isolated from mycobacterial culture, and PCR confirmation revealed presence of Maa.

Conclusion
Based on these findings, a diagnosis of granulomatous enteritis due to Maa was concluded.
To the authors' knowledge, this is the first case of Maa infection in alpaca in Europe. Maa should be included as possible cause of subacute-chronic diarrhoea in camelids.

POSTER ABSTRACTS

Veterinary Pathology: Exotic, wildlife & zoo animals

287 | NEUROLOGICAL LESIONS IN CERVIDS: A STUDY IN PORTUGAL

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Background
Brain samples were collected within the project “CWD Risk in Portugal”, profiting the infectious diseases epidemiological surveillance and screening of chronic wasting disease (CWD) during cervids hunts. The literature on wild animals´ neuropathological lesions is scarse. This work aims to describe the neuropathological findings in samples collected in deer hunted in Portugal.

Materials & Methods
For 4 years, 233 brainstem and cerebellum samples were collected from deer (97) and fallow deer (136) for routine histopathology analysis.

Results
In 15% of the samples, the diagnosis was impaired by autolysis. On the remaining samples, one choroid plexus papilloma was diagnosed. In 7.5% of cases, nonspecific-inflammatory changes were detected in the CNS, featuring a discreet perivascular lymphoplasmacytic infiltrate. A lymphoplasmacytic meningitis with rare eosinophils and histiocytes was also diagnosed, associated with nematodes’ presence in the meninges. In 20% of the cases, findings devoided or of uncertain pathological significance (no significance) were detected (neuronal lipofuscin deposits, polyglucosan bodies, neuronal hyaline inclusions, reactive astrocytes in white matter; mineralization of the vascular endothelium and melanosis in the meninges).

Conclusion
This exploratory study represented the first survey of neuropathological changes in wild cervids in Portugal, revealing mainly discrete non-specific inflammatory lesions, changes of little clinical significance, in addition to one neoplastic lesion. This approach should be expanded as an important tool to identify putative neuropathological zoonotic pathogens in the cervid population for early warning systems in the health surveillance of wildlife and humans as they are intended for consumption.

POSTER ABSTRACTS

Veterinary Pathology: Exotic, wildlife & zoo animals

292 | LESIONS OF ORAL CAVITY IN GREY SEALS HALICHOERUS GRYPUS

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Background
The health of jaws and oral cavity is important for the survival and population density of grey seals. Various environmental factors, including pollution, affect the structure of the bones and teeth. The aim of the study was to examine the oral cavity of grey seals for changes.

Materials & Methods
A gross examination of the oral cavity and body parameters (weight, size, sex, blubber thickness) was carried out on 53 wild grey seals found dead in fishing nets in the Gulf of Riga. Radiographic analyses of the bone structures of jaws were carried out. A canine tooth was removed from each seal to determine age based on the cementum layer.

Results
Various pathological changes were observed in 34% of the seals, such as tooth fractures, mandibular deformities, periodontitis and gingivitis. There was no association between the seals' blubber thickness and the development of these oral pathological changes ($p>0.05$). Significantly more seals (71.7%) exhibited tooth wear, which increased with age ($p<0.05$). The most pronounced teeth wearing was observed on incisors in grey seals reaching 15 years of age (58.5%), but first appearance of mild wearing was found in incisors in 5 y.o. (median) seals.

Conclusion
This study found that a substantial portion (34%) of grey seals have various oral pathological changes. These issues did not appear to be affected by body fat condition of the seals, nor did the body fat condition have any influence on the development of oral pathological changes. Tooth wear was a common finding (71.7%) that developed with age.

POSTER ABSTRACTS

Veterinary Pathology: Laboratory animals

10 | PATHOLOGY AND INNATE IMMUNE RESPONSE CORRELATES IN INFLUENZA A VIRUS VACCINATION-CHALLENGE MODEL IN DOMESTIC PIGS

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Background
Influenza A virus (IAV) poses a continual global health threat, underscoring the necessity for effective vaccines that offer broad immunity coverage. While IAV vaccines are effective in mitigating pulmonary pathology, studies have reported that inactivated formulations can induce vaccine-associated enhanced respiratory disease (VAERD) in experimentally infected pigs.

Materials & Methods
To assess the protective efficacy of a whole inactivated virus (WIV) vaccine against a 2009 pandemic strain of H1N1 virus, the trachea, lung, and tracheobronchial lymph node (TBLN) sampled from vaccinated and unvaccinated domestic pigs euthanised before challenge and 5 days post-viral infection were evaluated by histopathology, viral immunohistochemistry, and *in situ* hybridisation targeting porcine Mx1, CCL2, and TNF-α transcripts.

Results
The severity of bronchointerstitial pneumonia and viral antigen loads was reduced in vaccinated pigs compared to unvaccinated pigs. While Mx1 and CCL2 mRNA typically co-localised in areas with bronchointerstitial pneumonia, the transcript labelling were more abundant and prominent in unvaccinated pigs. Similarly, there was increased Mx1 and CCL2 labelling in the TBLN. However, there were no significant differences in TNF-α expression in the lung between vaccinated and unvaccinated pigs following viral challenge, nor Mx1, CCL2 and TNF-α in the trachea after viral challenge.

Conclusion
The WIV vaccine reduced pulmonary lesions and innate immune gene transcription during the acute stage of IAV infection, with no evidence of disease enhancement in the vaccination-challenge model of domestic pigs.

POSTER ABSTRACTS

Veterinary Pathology: Laboratory animals

118 | HISTOPATHOLOGIC CHARACTERISATION OF TUMOURS ARISING FROM A P53 KNOCKED-OUT MOUSE MODEL

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Background
p53 KO mice (p53-/- and/or p53+/-) are being explored by regulatory agencies to help reduce animal use and duration of rodent carcinogenicity studies. Homozygous p53 mice show a high susceptibility to cancer at younger ages already resulting in a potentially more rapid tumorigenesis induction following treatment, which can help save time and resources. In this study, we aimed to characterize histologic features of the tumours arising from different tissues in a p53-/- mouse models using H&E staining and Immunohistochemistry for Ki-67, CEA, CD3, PAX5, Pan-cytokeratin, AFP and vimentin.

Materials and Methods
During necropsy from 9 mice, the tissues suspected to be tumors in gross examination were sampled and fixed in 10% neutral buffered formalin. The isolated samples were stained by H&E and selected antibodies for Ki-67, CEA, CD3, PAX5, Pan-cytokeratin, AFP and vimentin antibodies.

Results
The average age of animals at the time of euthanasia was 160 days. Of the 9 animals, 8 animals had at least one organ developing a tumor. Three animals developed tumors from more than one organ. The tumors were developed in the skeletal muscles (rhabdomyosarcoma, n=3), spleen (T-Cell lymphoma n=4), liver (hepatocellular carcinoma, n=1) and lymph nodes (T-cell lymphoma, n=2)

Conclusions
This mouse model can be used in carcinogenicity studies as they developed different types of tumours spontaneously and rapidly.

POSTER ABSTRACTS

Veterinary Pathology: Laboratory animals

135 | SAFETY HISTOPATHOLOGICAL ANALYSIS OF ADMINISTRATION ROUTES OF TAMOXIFEN IN THE MURINE MODEL

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Background
Tamoxifen is wildly used as a lipophilic gene expression controller molecule in murine models. Its administration requires a dilution in oil or ethanol. Studies have shown the efficacy of the gavage, transdermal, subcutaneous and intraperitoneal injection routes, the last one being the most often used. This study aimed to compare the histopathological safety of gavage, transdermal, subcutaneous and intraperitoneal injection routes.

Materials & Methods
Forty-four 2-4 months female and male C57Bl/6N mice were administered 3 times over a week corn oil with or without Tamoxifen either by gavage or subcutaneous (SC) or intraperitoneal (IP) injection (n=4-5 each) or with ethanol with or without Tamoxifen by transdermal application (TD, n=4 each). Ten equivalent mice were administered saline by gavage or subcutaneous or intraperitoneal injection (n=3-4 each). At necropsy, administration sites were sampled and routinely embedded in paraffin. 3-4 µm slides were routinely stained with HES.

Results
Oil remnants were present 10 days after the last SC or IP injection of oil only or oil and Tamoxifen. It elicited a mild to marked granulomatous inflammation in the subcutaneous tissue and in the parietal and visceral peritoneum respectively. Few mononuclear inflammatory cells were noted in the gastric mucosa when administered oil only (2/4 samples). No relevant microscopic lesions were observed in the TD or saline administration groups nor in the oil and tamoxifen gavage group.

Conclusion
From a safety point of view, transdermal and gavage administration routes of Tamoxifen should be preferred compared to subcutaneous and intraperitoneal injections in the murine model.

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Veterinary Pathology: Laboratory animals

149 | PATHOLOGY AND HOST-PATHOGEN INTERACTION IN A HAMSTER MODEL OF NIPAH VIRUS INFECTION

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Background
Nipah virus (NiV) is recognised as one of the key pathogens with pandemic potential. We have recently developed a NiV hamster model, characterised by respiratory and neurological disease, mimicking human infection.
The aim of this study is to investigation the host-pathogen interaction of NiV infection, focusing on the proinflammatory immune response which takes place in the lung and brain.

Materials & Methods
28 animals were infected with NiV Malaysian strain and euthanised at different time points. The animal experiment was approved by the UKHSA AWERB Committee. Lung and brain FFPE samples were selected for histopathological evaluation (H&E) and RNAscope ISH to detect NiV RNA and cytokine mRNA (IL6, TNF and IFNβ1). IHC was used to mark T cells ($CD3^+$), macrophages/microglia (Iba1) and astrocytes (GFAP). Multiplexed IF (NiV antigen, CD3, GFAP and IBA1 antibodies) was also used.

Results
Lesions consisted of moderate to severe multifocal broncho-interstitial pneumonia accompanied by mild meningitis and perivascular cuffing in the brain. NiV RNA was detected in endothelial cells and inflammatory cells within lesions. Abundant $IBA1^+$ cells were detected in the areas of broncho-interstitial pneumonia, together with $CD3^+$ cells, mainly perivascular. In the brain, abundant $IBA1^+$, $CD3^+$ and $GFAP^+$ cells were present in cases with more severe neurological damage. Upregulation of proinflammatory cytokines was observed within the lesions present in lung and brain.

Conclusion
These results have helped us to characterize the host-pathogen interaction in the hamster animal model of NiV infection, that is being currently used in preclinical testing of antiviral and vaccine strategies.

POSTER ABSTRACTS

Veterinary Pathology: Laboratory animals

174 | SUBTYPING OF MOUSE PANCREATIC DUCTAL ADENOCARCINOMA (MPDAC)

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Background
Mouse pancreatic ductal adenocarcinoma (mPDAC) models are often used to reproduce human PDAC (hPDAC) and its progression. Identifying mPDAC subtypes comparable to humans is crucial for the proper translation of findings within model systems. One mesenchymal (C1) and three epithelial (C2a, C2b and C2c) mPDAC transcriptomic subtypes were previously identified. The project aims to detect immunohistochemical surrogate markers to differentiate the epithelial subtypes on histological slides.

Materials & Methods
Based on RNA sequencing data from 38 mPDAC cell lines generated from mouse models with PDAC, 28 markers were analysed immunohistochemically on cell pellets of mPDAC cell lines of known subtype (n=20). The percentage of positive tumour cells and staining intensity were investigated semi-quantitatively. The markers were further tested on 32 orthotopic transplant tumours of the respective cell lines.

Results
In the cell pellets, only 2 of 28 markers, Necdin (NDN) and Placenta associated 8 (PLAC8), showed differential staining of the epithelial subtypes. Transplant tumours of subtypes C2b and C2c stained strongly for NDN, and at least 20 % of these tumour cells were moderately or strongly positive for PLAC8. No or less than 20 % of the neoplastic cells were stained with the same intensity for both markers in the C2a subtype.

Conclusion
Our preliminary results indicate that NDN and PLAC8 may be potential surrogate markers for differentiating mPDAC subtypes C2a from C2b or C2c. Further investigations in endogenous mPDAC tumours have yet to validate their suitability, but they might enable further translational characterisation of mPDAC compared to hPDAC.

POSTER ABSTRACTS

Veterinary Pathology: Laboratory animals

251 | RHABDOMYOSARCOMAS IN DYSTROPHIN-DEFICIENT SPRAGUE DAWLEY RATS

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Background
Duchenne muscular dystrophy (DMD) is one of the most common muscular dystrophy in humans, mainly affecting boys with a mutation in the large *Dystrophin* gene (*DMD*). Animal models of DMD exist in various species with the most commonly described being mice and dogs. Transgenic DMD rats harbouring a deletion within the exon 52 of the *Dmd* gene (R-DMDdel52 rats) have been recently created on a Sprague Dawley background. Here, we describe the characteristics of spontaneous tumours arising in this model.

Materials & Methods
R-DMDdel52 male rats with palpable tumour were selected and the localisation was recorded. Tumours were sampled for cytopathology, histopathology, immunofluorescence for TRPS1, PAX7 and myogenin, and immunohistochemistry for myogenin, desmin and vimentin.

Results
Forty-three tumours were collected. Affected rats were 7 to 15 months old (mean age = 12 months). Most tumours were located to the head and neck (32/43), mainly affecting masseter muscles (26/32). Cytological and histological features were consistent among cases with highly cellular tumours made of undifferentiated round to pleomorphic spindle cells with variable number of multinucleated cells. On immunofluorescence, all tested tumours were positive for PAX7 and TRPS1, 90% were positive for myogenin. On immunohistochemistry, one tumour was tested and was positive for myogenin, desmin and vimentin. Based on these features, rhabdomyosarcomas (RMS) with morphological characteristics of pleomorphic RMS were diagnosed.

Conclusion
RMS spontaneously develop in R-DMDdel52 male rats at a high incidence whereas these tumours are exceedingly rare in children with DMD. R-DMDdel52 rats could represent a new model for RMS.

POSTER ABSTRACTS

Veterinary Pathology: Laboratory animals

262 | DYNAMIC CHANGES OF ASTROGLIAL REACTIONS IN AN INFECTIOUS MODEL OF MULTIPLE SCLEROSIS

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Background
Intracranial infection of SJL mice with Theiler's murine encephalomyelitis virus (TMEV) induces an acute polioencephalitis and chronic demyelinating leukomyelitis, which is used as an animal model for human multiple sclerosis. Astrocytes, which are a major target cell of TMEV, are involved in the maintenance of blood-brain-barrier integrity, synapse formation and the propagation of action potentials as well as pro- and anti-inflammatory functions.

Materials & Methods
The spinal cord of TMEV-infected SJL mice were investigated by light microscopy and immunohistochemistry to characterize astroglial reactions at 14, 42, 98, 147, 196, and 245 days post infection. Furthermore, the expression pattern of astrocytic genes in the spinal cord during the progression of demyelination was analyzed using microarray data.

Results
TMEV infection resulted in a continuously increasing inflammation, demyelination and astrogliosis in the spinal cord, which was associated with an increased protein expression of ALDH1L1, GFAP, S100A10 and vimentin. Microarray analysis demonstrated an upregulation of genes mediating neuroinflammation (A1) and genes related to reparative functions (A2) of astrocytes.

Conclusion
The activation of astrocytes correlates with the disease course of TMEV infection and leads to the production of neurotoxic and neuroprotective factors. The expression of selected candidate genes will be analyzed in detail to decipher the role of astrocytes in the initiation and progression of TMEV-induced demyelination disease.

POSTER ABSTRACTS

Veterinary Pathology: Others

58 | MACROSCOPIC, HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL DESCRIPTION OF RECURRENT TRAUMATIC LESIONS IN A NINE-MONTH OLD CAT: A FORENSICS CASE

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Background
A forensic necropsy was required by the Police on a nine-month old female cat after the owner's neighbours filed a complaint against him for suspicion of abuse.

Materials & Methods
A complete necropsy was performed and samples from several organs including traumatic lesions were taken for histological (HE&S, Perls, Masson's Trichrome stainings) and immunohistochemical (GFAP - Glial Fibrilliary Acidic Protein for astrocytes - and Iba-1 - Ionized Calcium-binding Adapter Molecule 1 for microglial cells) analysis.

Results
At necropsy, numerous traumatic lesions were observed such as fractures, hematomas, pneumothorax, pulmonary contusions etc. Some of them, in particular on the dorsal aspect of the head and in the perineal area, presented with both acute and chronic characteristics. In these two regions, subcutaneous tissues were congested, edematous and haemorrhagic but also emphysematous and necrotic as well as thickened and firm. At histopathology, these lesions were composed of hemorrhages, edema, bacterial colonies and fibrin deposits amongst immature and mature fibrous tissue comprising siderophages. The cerebral cortex presented with histological and immunohistochemical changes compatible with "chronic traumatic encephalopathy" such as a multifocal spongiosis of the most superficial cerebral cortex areas. In these areas, astrocytes and microglial cells presented with a modified appearance, respectively highlighted by GFAP and Iba-1 immunohistochemistry, from a "quiescent" state to an "activated" state.

Conclusion
This case describes traumatic lesions with both recent and ancient characteristics, evocating recurrent injuries over a course of at least several weeks.

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Veterinary Pathology: Others

76 | SPONTANEOUS HEPATOCELLULAR AND GASTRIC TUMORS IN AFRICAN CLAWED FROGS (XENOPUS LAEVIS)

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Background
Three 7-yr-old African clawed frogs (*Xenopus laevis*) showed weight loss, celomic distention and an abnormal swimming gait, and were euthanised on welfare grounds.

Materials & Methods
A complete necropsy and histopathology was performed on each animal. To immunophenotype the hepatic and gastric masses, immunohistochemistry using common antibodies in oncopathology was performed: cytokeratin AE1/AE3, vimentin, E-cadherin, P53 and Ki67 and, additionally, for the hepatic tumors only, synaptophysin, Prox-1, S100 and Sox-9.

Results
Upon postmortem examination 2 animals showed a focal, firm, dark brown to green mass with multifocal haemorrhages in the left liver lobe whereas another frog showed a transmural, firm, beige, multilobulated gastric mass. Additionally, one of the frogs with the hepatic mass had hemoceloma. Histologically, the hepatic masses corresponded to non-encapsulated, multilobulated, expansile hepatocellular neoplasms (hepatocellular adenomas) with variable sinusoidal ectasia. The gastric mass histologically corresponded to a transmural, densely cellular, infiltrative, malignant epithelial neoplasm (gastric carcinoma). Trans-celomic metastases were observed. One of the hepatocellular adenomas was weakly positively labelled with E-cadherin whereas the other showed variable positivity for Sox-9 only. Vimentin labelled the stroma and sinusoidal endothelia. Interestingly in the liver, the cytokeratin AE1/AE3 labelling was restricted to the biliary epithelium and sinusoidal endothelia. The gastric carcinoma labelled positively with cytokeratin AE1/AE3 only.

Conclusion
This report aims to guide laboratory animal veterinarians to accurately diagnose multi-organ masses in amphibians. To the authors' knowledge, this is the first comprehensive morphological study on a case series of hepatocellular and gastric neoplasia in *Xenopus laevis*.

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Veterinary Pathology: Others

123 | SALIVARY PLEOMORPHIC ADENOMA IN AGED RHESUS MACAQUES (MACACA MULATTA)

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Background
Pleomorphic adenomas are common benign neoplasms in the salivary glands of humans, primarily affecting the parotid gland. They often recur but rarely metastasize. Salivary neoplasms are rare in non-human primates (<1% frequency). We report three spontaneous pleomorphic adenomas in aged rhesus macaques.

Materials & Methods
Three male rhesus macaques (*Macaca mulatta*), aged 24 to 30 years old, were necropsied for unrelated reasons. The parotid glands of two animals and the submandibular gland of the third animal contained a mass that was processed for histopathology. Immunohistochemistry for pancytokeratin and smooth muscle actin (SMA) were performed in two cases.

Results
Grossly, the salivary gland parenchyma was compressed by 2x1x1 cm, round to oval, firm, mottled tan to grey nodules with small foci of necrosis. Histologically, the nodules comprised an encapsulated neoplasm composed of a dual population of epithelial and myoepithelial cells organized in nests, tubules, and ducts on a myxomatous to hyalinized stroma. Anisocytosis and anisokaryosis were moderate, and the epithelial component had 1-3 mitotic figures in 2.37 mm^2. Cytoplasmic pancytokeratin immunoreactivity was strong in 100% of the epithelial population, weak in 20-30% of the myoepithelial cells, and absent in the stroma. Cytoplasmic SMA immunoreactivity was strong in 100% of the stromal population, weak in 10-20% of the myoepithelial cells, and absent in the epithelial cells. The dual proliferation of epithelial and myoepithelial cells indicates a diagnosis of pleomorphic adenoma.

Conclusion
These findings support the diagnosis of pleomorphic adenoma in aged rhesus macaques and is the first description in nonhuman primates.

POSTER ABSTRACTS

Veterinary Pathology: Others

161 | HISTOPATHOLOGICAL STUDY OF DISEASED SEA URCHINS FOUND DEAD IN A ROCKY BEACH OF GRAN CANARIA (CANARY ISLANDS)

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Background
Sea urchin diseases have been reported around the world, associated to mass mortality produced by bacteria, protozoa, fungi and algae. However, there is still controversy because many of these pathogens are also found on healthy specimens. The objective of this work was to perform the pathological study to explore the potential correlation of histological lesions with pathogens in diseased sea urchins in Gran Canaria.

Materials & Methods
Diseased and apparently healthy individuals of sea urchins (*Arbacia lixula* and *Paracentrotus lividus*) were collected in the months of June, July and October 2022. Samples were taken from the external and internal face of lesions, scraping the test area with a sterilized swab, and in the external and internal face of the healthy test sea urchins. Samples were incubated using different culture media and strains were identified by mass spectrometry. In addition, samples of the lesions of diseased and healthy individuals were fixed in formaldehyde. STest samples were decalcified for 5 minutes.

Results
Gross findings in both sea urchin species were bare test surfaces, without spines or pedicellaria, and discoloration of the test. Histologically, at the edge of the injured areas an inflammatory reaction composed mainly of coelomocytes and pigment cells was observed. Additionally, in the injured areas gram-negative bacterial colonies were detected. *Vibrio alginolyticus* was the most frequent bacterium in diseased specimens of both species.

Conclusion
This study establishes a histopathology association between the presence of bacterial colonies in the diseased sea urchins and an accompanying inflammatory response in the affected tissues.

POSTER ABSTRACTS

Veterinary Pathology: Others

167 | PATHOLOGICAL STUDY OF A GAS BUBBLE DISEASE MODEL IN FISH

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Background

Gas bubble disease (GBD) occurs due to the supersaturation of water by dissolved gases, leading to the development of gas bubbles in the circulation and extravascular tissues. An experimental model of GBD in fish could be useful to study the pathophysiological processes triggered by the presence of gas bubbles in the organism, being applicable to deepen the understanding of GBD and, transversely, to other conditions such as decompression sickness. The pathological study is crucial to validate this experimental model.

Materials & Methods

A low-pressure vessel coupled to a pressurized aquarium was designed to produce supersaturated water. Pressurized air was injected and forced into dissolution by constant recirculation of the water. Goldfish (*Carassius auratus*) (n=12) were individually introduced in the pressurized aquarium and exposed to 112±5% supersaturation for 18 hours. The control group (n=12) was introduced in non-supersaturated water. Clinical signs were constantly monitored. All fish were euthanized after 18h and a pathological study was performed, including a gas score method.

Results

GBD group showed clinical signs consistent with severe GBD such as erratic movements and loss of buoyancy, with external lesions such as multifocal hemorrhages, congestion, and emphysema of fins. Massive presence of gas bubbles systemically distributed with a high gas score and associated lesions such as hemorrhages and congestion affecting gills, heart, and posterior kidney, among others were observed.

Conclusion

These results validate this GBD model in fish as a novel alternative model to study further the pathophysiological pathways activated by gas bubbles in the organism.

POSTER ABSTRACTS

Veterinary Pathology: Others

182 | HISTOPATHOLOGICAL FINDINGS RELATED TO PROTOZOAN PERKINSUS INFECTION IN MANILA CLAMS (RUDITAPES PHILIPPINARUM)

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Background
Manila clam is a bivalve mollusk with high commercial value. Their aquaculture productivity in coastal lagoons is endangered by a complex interplay of environmental factors. *Perkinsus* sp alveolate protozoan dinoflagellates are among aetiologic agents affecting clams health . In Italy, perkinsosis is a cryptic disease in clams, with a wide range of prevalence. The parasite is known to rely on iron for proliferation and infection. The aim of this investigation was to describe the morphological aspects of *Perkinsus* infection and to assess iron content and evaluate its role in host-parasite interaction.

Materials & Methods
Thirty-five clams were provided by a commercial trader of Northern Italy; animals were anaesthetized by immersion in ethanol and whole bodies removed from the shells. From FFPE embedded blocks, consecutive sections were stained with H&E, PAS, Perls, and Lillie to emphasize host-produced material and iron content.

Results
Ten out of 35 clams displayed trophozoites of *Perkinsus* sp.; gills' connective tissue was the most commonly affected, followed by gonads and digestive glands. Tissue reaction was characterized by haemocyte recruitment, granulocytomas formation and presence of thick layer of PAS-positive material surrounding trophozoites. Perls stained ferric iron content within both trophozoites and haemocytes.

Conclusion
Distribution and type of host reaction mirrored the descriptions of *Perkinsus* sp. Infection in the literature. Although proteomic profiles of iron-acquisition systems of *Perkinsus* sp. are well known, we characterized the features of the infection describing the presence of intraparasitic and intrahaemocytic ferric iron, suggesting possible iron-competing mechanisms between the host and the parasites.

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Veterinary Pathology: Others

188 | DETERMINATION OF EARLY POST-MORTEM INTERVAL IN SWINE VIA ELECTRICAL MUSCLE STIMULATION

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Background
The early post-mortem interval (PMI) is classically estimated using post-mortem changes such as rigor mortis and livor mortis. However, this is often imprecise and further techniques are used in human forensic medicine to improve the determination. This study aimed to determine the early PMI through electrical muscle stimulation (EMS).

Materials & Methods
Four different groups of striated muscle (from the extraorbital eyelid, snout, cutaneous trunk and anal sphincter) of eight porcine carcasses with known time of death were stimulated using the electric stimulation device (MD95/2007 Funeralia®) consisting of puncture electrodes with 40V output. The time dependent reaction to EMS was recorded and a grading system of the reaction was established for each muscle group.

Results
The muscle groups showed a time-dependent decrease in excitability. The complete loss of muscle reaction to EMS differed in time between the analyzed muscle groups. The longest PMI with up to six hours was measurable in the muscle of the anal sphincter.

Conclusion
The EMS appears to be a viable method to determine the early PMI up to six hours after death with a simple and ready-to-use device more precisely.

POSTER ABSTRACTS

Veterinary Pathology: Others

193 | ASSOCIATION OF AEROMONAS SPP. WITH MORTALITY AND PERIVISCERAL ADIPOSE TISSUE NECROSIS IN REDTAIL CATFISH (PHRACTOCEPHALUS HEMIOLIOPTERUS)

L. Caballero-Hernández[1], A. Castro-Alonso[1], M.J. Caballero[1], Á. Curros-Moreno[2,3], G. Montero-Hernández[1], D. Padilla[1], L. Marrero-Ponce[1], E. Montesdeoca-Morales[1]
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Background
Aeromoniasis is a disease caused by different bacteria belonging to the genus *Aeromonas*, and it is known to have high mortality rates in fish. The objective of this study is to describe the lesions associated with these bacteria in a group of redtail catfish (*Phractocephalus hemioliopterus*) from a private aquarium.

Materials & Methods
Necropsies were performed on seven redtail catfish (*Phractocephalus hemioliopterus*) that suffered from acute deaths. Samples were collected for bacterial culture and histological examination of all organs.

Results
The fish presented with non-specific clinical signs of sudden onset on the day of death, including erratic swimming. External examination did not reveal typical *Aeromonas* lesions, such as skin haemorrhages, only some fish presented with distention of the coelomic cavity. Internally, a substantial amount of perivisceral fat was observed in the coelomic cavity with extensive multifocal flattened lesions, diffuse borders and yellowish-reddish colouring. Histologically, the adipose tissue showed extensively vascularised septa of connective tissue infiltrated by a dense mass of macrophages containing a fine to coarse brownish-yellow granular material. In addition, a severe cellular inflammatory reaction and multifocal necrotic areas were observed. Bacterial culture and PCR were positive for *Aeromonas veroni* and *Aeromonas hydrophila* in the adipose tissue samples.

Conclusion
This work describes for the first time the association between *Aeromonas* spp. and extensive multifocal perivisceral adipose necrosis in acute deaths of redtail catfish maintained under human care.

POSTER ABSTRACTS

Veterinary Pathology: Others

213 | HAVE YOU HEART THE NEWS? INCLUSION BODIES IN ATRIAL CARDIOMYOCYTES OF GUINEA PIGS MAY SURPRISE YOU

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Background
Cardiomyocytes of guinea pigs are full of surprises, since another type of intracytoplasmic inclusions than the recently described protein aggregates, was incidentally identified on post-mortem routine examination.
This study was conducted to assess their incidence, their morphological, staining, and ultrastructural characteristics, and their pathological significance.

Materials & Methods
Retrospective necropsy cases from 2014 to 2023 with atria sampled for histopathological examination were included. Clinical and necropsy data were collected. An image analysis software was used to evaluate the inclusions burden on digitized PAS-stained atrial sections. PAS-amylase, mucicarmine, Gomori-Grocott's metamine silver and Alcian blue pH 2,5 were performed on 3 cases and transmission electron micrographic (TEM) examination on 2 cases.

Results
Inclusion bodies were found in 20 of 20 guinea pigs. The inclusions were numerous in the right atrium (6 ± 2 inclusions/mm²), rare in the left atrium (1 ± 1 inclusions/mm²) and were preferentially localized in the subendocardial region. They were intracytoplasmic, ovoid to linear, frequently fragmented, slightly basophilic in HES-stained sections and range from a few to more than 200 µm. They stained positively to PAS, mucicarmine, Gomori-Grocott's metamine silver and Alcian blue pH 2,5 and were amylase resistant. TEM revealed a non-membrane-bound slightly electron-dense fibrillar material. They seemed to be less frequent in young animals and were not associated with sex, local inflammation and cardiac or systemic disease.

Conclusion
This study presents amylase-resistant PAS-positive intracytoplasmic inclusions in atrial cardiomyocytes of guinea pigs suspected to result from an age-related disorder in the carbohydrate metabolism, with no clinical consequences.

POSTER ABSTRACTS

Veterinary Pathology: Others

224 | 5-HT-2B RECEPTORS AND ASTROCYTES CROSSTALK IN KETAMINE ANTIDEPPRESSIVE ACTION

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Background
The potential of the anaesthetic ketamine in the treatment of depression has been raising attention. The 5-HT-2B serotonin receptor is a therapeutical target for selective serotonin antidepressants and it is expressed in neurons and astrocytes. Astrocytes have been described to play a key role in the pathophysiology of depression. Given the emerging understanding of 5-HT-2B expression in zebrafish, it's pertinent to assess its resemblance to astrocyte distribution and any alterations following ketamine exposure.

Materials & Methods
Zebrafish embryos at the 1-4 somites stage (10.5 hours post fertilization) were randomly distributed into treatments: control or exposure to ketamine concentrations of 0.2 or 0.8 mg/mL for 20 min. Seven months later, brains were collected to study immunoexpression of 5-HT-2B and glial fibrillary acidic protein (GFAP), a marker for astrocytes, or to quantify 5-HT-2B receptors by western blot (WB) (n= 3).

Results
5-HT-2B immunoexpression was observed in non-identifiable cell types in the optic tectum, torus longitudinal, medulla oblongata and vagal lobe. Similarly, GFAP immunoexpression was high in the hindbrain area, namely in the medulla oblonga and the vagal lobe with no apparent differences among groups. Nonetheless, 0.2 mg/mL of ketamine downregulated the 5-HT-2B receptor levels (p=0.04).

Conclusion
The reduced levels of 5-HT-2B receptors in the group exposed to 0.2 mg/mL suggest their importance in ketamine's antidepressant effects in zebrafish. Since 5-HT-2B receptors and GFAP are found in the same brain regions this model indicates astrocytic and neuronal crosstalk in ketamine's therapeutic action. Further co-localisation studies are needed to confirm receptor expression sites

POSTER ABSTRACTS

Veterinary Pathology: Others

282 | LIMITED EVIDENCE OF THE EFFECTIVENESS OF ANTIVIRAL DRUGS TO PREVENT BRAIN INFECTION BY SARS-COV-2 IN THE K18-HACE2 MURINE MODEL

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Background
Neurological impairments in patients with COVID-19 suggest that SARS-CoV-2 can affect the central nervous system, although direct infection remains debated. Using the K18-hACE2 mouse model, in which virulent SARS-CoV-2 cause widespread brain infection after intranasal challenge, we investigated whether antiviral drugs can block brain infection.

Materials & Methods
K18-hACE2 mice were treated with known antivirals (prior to and after intranasal SARS-CoV-2 infection) of different modes of action: a) direct interference with RNA dependent RNA polymerase (RdRP) activity (remdesivir; intraperitoneal), b) RNA mutagenesis leading to catastrophic errors in viral genome (molnupiravir; peroral), c) inhibition of main viral protease (nirmatrelvir; peroral), and d) PIKfyve phosphoinositide kinase inhibitor (apilimod dimesylate; intranasal). Mice were infected with either SARS-CoV-2 Pango B (10^4 PFU) or Delta (10^2 or 10^3 PFU) and the brains examined by immunohistology for SARS-CoV-2 nucleocapsid protein (NP) between 4 and 7 days post infection (dpi).

Results
Brain infection was detected in similar proportions of untreated mice and those treated with remdesivir, nirmatrelvir or apilimod between 4 and 7 dpi. Only the mice treated daily with molnupiravir (1 day pre- until day 4 post-infection) with SARS-CoV-2 Pango B appeared not to be infected by 5 dpi.

Conclusion
Antiviral treatment based on different mechanisms does not seem to have any effect on the ability of SARS-CoV-2 to reach the brain. Interestingly, while apilimod should prevent productive neuronal infection, it did not prevent brain infection, maybe due to its intranasal application. Molnupiravir might have a preventive effect but this requires further investigation

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