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WELCOME FROM THE LOCAL ORGANIZING TEAM

Dear Colleagues,

We are pleased to welcome you to Bologna for the ESVP-ECVP Joint Meeting 2016.

A fascinating and diverse scientific programme covers multiple fields of pathology and is offered by keynotes, workshops, slam, oral and poster presentations. Social activities centre on the annual meetings of the ESVP and ECVP and culminate in the Social Dinner. We are sure that during the meeting you will enjoy an active interchange of your scientific work with other colleagues and the opportunity to build new collaborations.

A special thank you to all the invited speakers who are giving state of the art presentations and to all of you who have submitted the abstracts that make the meeting a success. The presence of more and more colleagues from across the world at Congress each year emphasizes the international nature of our meetings. Thank you all!

A special thank to Germana, Valeria, Luisa and Michela who contributed so much time and energy to help with the organisation of the meeting and to Dr. Roberta Pasquini of MV Congressi Spa for her superb supervision.

Bologna is a historic city at the heart of Europe, rich in Churches, Museums, Towers and is celebrated for its gastronomy. So we warmly suggest that you visit the city, see its sights and taste its food, of which you can have an initial feeling during the social dinner.

Thank you for your presence and we wish you a fruitful meeting and a memorable stay in Bologna.

Giuseppe Sarli, Cinzia Benazzi, Barbara Brunetti, Giancarlo Avallone

WELCOME FROM THE ESVP AND ECVP PRESIDENTS

Dear Colleagues

It is with great pleasure that we welcome you to the annual joint ESVP-ECVP congress in beautiful Bologna, the town with the oldest Western University.

This year's congress covers a range of topics of great interest. The key note lectures and several sessions give emphasis to infectious diseases and the One-Health initiative. You will also find a dedicated lymphoma session, workshops on skin and renal pathology, a neuropathology mini-symposium, and the well-established Mystery Case session. The programme is complemented by a specific session on the approach to peer reviewing and the "Tips and tricks for exam preparation" session for trainees. Together with the Scientific Committee and Local Organising Committee, we are also trying new types of presentations and discussions, like a poster flash and slam sessions. These will be an interesting experience and we are looking forward to your feedback comments.

This is the 27th joint annual meeting of the society and college which could not take place without the dedication and motivation of the Local Organising Committee. We therefore use the opportunity to thank Professors Cinzia Benazzi, Barbara Brunetti and Giuseppe Sarli and Dr. Giancarlo Avallone for organizing the meeting in Bologna. The joint responsibility of ESVP and ECVP for the annual meeting is also logistically reflected in the contribution of the Scientific Committee and we would like to take to opportunity to thank all members of the committee for their outstanding and time-consuming efforts.

The willingness of local colleagues to dedicate time and effort into the organization of these events give us all the unique opportunity to

become more acquainted with the scientific landscape across Europe in our area and showcase specific local expertise, also reflecting the diversity of our profession. The Scientific Committee ensures the high scientific standard and relevance of the programmes, making our annual meeting an extremely valid CPD event. We are aware that it is a challenge to assemble a cutting edge, balanced programme that is of interest for the entire profession, since veterinary pathologists work in various fields, in particular also in their research, while being only a relatively small group. Hence, in a joint effort, society and college have recently developed new standard operating procedures (SOPs) for both the Scientific Committee and local organisers. These will make the work in particular of local organizing teams much easier and will ensure consistency. Please have a look at the SOPs at the members' area of the website if you are considering organising one of our future annual meetings.

Both society and college will hold their annual general meetings during the conference. We encourage you all to attend and engage more with your professional bodies in particular in these exciting times, when we have started to work together much more closely.

During your stay in Bologna you may want to visit some of the beautiful historic places, such as the Palazzo Poggi, the seat of the present University, or some of the University Museums. Of particular interest to all of us should be the Anatomical Theatre, where practical teaching of cadaver dissections took place. The beautiful locations chosen for the social programme represent the perfect environment to meet colleagues and friends and "network". It might help you find your next job or embark on a research collaboration, just to name a few options.

We encourage you to make the best of the next few days and enjoy both the science and the social programme. It is thanks to the local organisers that we will all participate in a memorable event. Thank you for your support and have a great time.

Anja Kipar
Prof. Dr.med.vet.habil., DiplECVP, FRCPath
President ECVP

Wolfgang Baumgärtner
Prof. Dr.med.vet.habil., PhD, DiplECVP
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Prof. Joaquim Segales

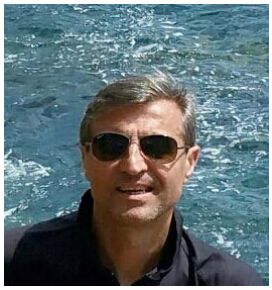
Emerging and re-emerging swine infectious diseases

Academic degrees: DVM (1991), PhD (1996), Dipl. European College of Veterinary Pathologists (ECVP, 2000) and Dipl. European College of Porcine Health Management (ECPHM, 2004, founding member)

Current position: Director and researcher of the *Centre de Recerca en Sanitat Animal* (CReSA) and Associate Professor at the Veterinary School of the *Universitat Autònoma de Barcelona* (main subjects: pathology and swine clinics). Vice-president (for the period 2010-13) and President (for the period 2013-16) of the ECPHM.

Veterinary service activity: Diagnostician at the Pathology Department of the Veterinary School of Barcelona since 1996. Responsible for the pathological diagnostic activity in swine (1996-2012).

Research activity: Involved in research of swine diseases since 1993, mainly infectious diseases (including infections by porcine reproductive and respiratory syndrome virus (PRRSV), Aujeszky's disease virus, porcine circovirus type 2 (PCV2), swine hepatitis E virus, swine Torque teno sus viruses (TTSuV), *Actinobacillus pleuropneumoniae*, *Haemophilus parasuis* and *Mycoplasma hyopneumoniae*). He has co-authored more than 250 articles in international peer-reviewed journals. Co-author of 3 patents (non-licensed) on PCV2 and TTSuV. He recently started working on MERS (Middle East Respiratory Syndrome)-coronavirus infection animal models.



Dr. Loris Alborali

Diagnostic protocols of infectious diseases in swine

DVM, ECPHM, Responsible of Animal Health Diagnostic Department, IZSLER, Brescia, Italy

G. Loris Alborali was born in Salò (Brescia), Italy. He obtained the DVM by the University of Milan in 1988. In 1989 he worked as veterinary practitioner and from 1991 he works as veterinary leader at Istituto Zooprofilattico Sperimentale Lombardia and Emilia Romagna (IZSLER) in Brescia. From 1991 to 1993 he worked in antigenic production and vaccine control Department and from 1993 to 1995 in farm epidemiology surveillance Department. From 1995 to date he works in Animal Heath Diagnostic Department and since 1999 he is the director of the Department. In 2009 he has board certified by the European College of Pig Health Management (ECPHM). Since 2013 he is the president of Italian Society of Pathology and Swine Farm (SIPAS). He works in veterinary service and in research activity. In pathological diagnostic activity in swine he spent the most part of time for support of field and to improve control and health management in respiratory, enteric and reproductive swine disease. The topic of research activity is swine disease, mainly infectious disease, biosecurity and monitoring program of bacterial diseases and antibiotic-resistance. He took part in national and international research project and biosecurity program.

EMERGING AND RE-EMERGING SWINE INFECTIOUS DISEASES

Joaquim Segales

IRTA, Centre de Recerca en Sanitat Animal (CReSA) and Dept. Sanitat i Anatomia Animals, Facultat de Veterinària, Campus de la Universitat Autònoma de Barcelona, 08193 Bellaterra (Cerdanyola del Vallès), Spain

Introduction: Emerging infectious diseases (EID) are those which incidence is increasing following its first introduction into a new host population or in an existing one as a result of long-term changes in its underlying epidemiology. This concept can also include those diseases linked to pathogens expanding into an area in which it was not previously reported, or due to pathogens that changed significantly its clinico-pathological presentation. The number of novel conditions in swine included under the concept of emerging and re-emerging diseases has increased importantly during last 20-30 years. Most of them are infectious diseases; their transmissibility and maintenance into a population is favoured by a number of phenomena, including intensive rearing practices and globalized/ international trading. The objective of this presentation is to discuss about new swine diseases or novel presentations of already known diseases, as well as newly recognized infections with a not well-defined pathogenic effect in pigs.

Monofactorial pig diseases: Traditionally, veterinarians have dealt with overt diseases, with the main task of counteracting them and getting profitability of the production system represented by a farm or a group of farms. Moreover, several decades ago, the most important diseases affecting pigs were considered mostly "monofactorial", in which the sole presence of the infectious agent was sufficient to trigger significant disease or production losses. In swine, most of these infectious diseases, such as classical swine fever (CSF), Aujeszky's disease (pseudorabies), foot-and-mouth disease or African swine fever (ASF), among others, have been controlled or are under control in many parts of the world by means of eradication programs (World Organization for Animal Health, OIE, www.oie.int). However, these diseases may appear sporadically in free-countries as a result of trading of life animals or animal products, or citizen travelling. This scenario of disease re-introduction is not unusual, and recent examples of emerging and re-emerging diseases would be ASF in Russia and Eastern Europe, and porcine epidemic diarrhoea (PED) in Europe and North-America.

Multifactorial pig diseases: There are a number of not so devastating infections compared to those considered as "monofactorial", but with significant impact on the economy of the swine industry. Among them we can consider those caused by *Mycoplasma hyopneumoniae, Brachyspira hyodysenteriae, Actinobacillus pleuropneumoniae*, porcine reproductive and respiratory syndrome (PRRS) virus (PRRSV), swine influenza virus (SIV) and porcine circovirus type 2 (PCV2). Common to all of them is that they are considered "multifactorial" diseases, since the mere presence of the agent is not sufficient to trigger the disease. In most of the cases, those diseases behave as endemic diseases in most parts of the world, but some exceptions can be perfectly classified as emerging or re-emerging pig diseases. Good examples would be the infection by certain serotypes of *A. pleuropneumoniae* in naïve herds or the reproductive form of PRRS in sero-negative farms, which may behave as the previously mentioned "monofactorial" diseases. Moreover, other diseases have probably been there for a long time, but their relevance increased mainly in the last two decades, such as colibacillosis, Glässer's disease, proliferative enteropathies, and swine influenza. Although the term "emerging" is probably old-fashioned nowadays for these conditions, we still consider PRRS and PCV2-associated diseases (PCVDs) as such. A good example of a truly emerging disease among these multifactorial threats would be Senecavirus A infection producing vesicular disease, a novel atypical porcine pestivirus related with congenital tremors, and *Brachyspira hampsonii*.

Zoonotic pig diseases/infections: The list of zoonotic agents that produce disease or infection in pigs is rather long, although in practical terms, just few of them are of significant importance. Moreover, the list of emerging zoonotic threats coming from pigs is rather limited, but the aware-

ness about them has increased during the last decade: hepatitis E virus (HEV), livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA), Extended-spectrum beta-lactamases (ESBL) producing bacteria, *Streptococcus suis, Clostridium difficile*, and *Cysticercus cellulosae*. There are also some pathogens that exerted their action in limited geographical locations, such as Nipah, Bungowannah, and Menangle viruses, that have been described as the cause of swine disease in certain regions of the world (south-east Asia and Oceania, mainly). Recently, the Middle East Respiratory Syndrome-coronavirus (MERS-CoV) has been shown to be able to infect pigs causing subclinical infection.

Discussion: Infectious emerging diseases appearance has been usually characterized by sudden, unpredictable outbreaks, sometimes of epidemic proportion. Despite improvements in global health, both at human and animal sides, outbreaks of infectious diseases still occur, and new infections have emerged and will probably continue emerging in the future. The control of a given disease/infection might be very difficult *per se*, and sometimes to live with an endemic scenario could be worse than to eradicate it and being threatened by the risk of re-infection. Such situation is especially important when dealing with livestock diseases/infections, since the loss of production or competitiveness may cause significant economic disadvantages, not only for the producers but also for a country or region as a whole. It is hard to predict what will come next in the swine industry in terms of diseases, but the advent of PRRSV by late 80s and beginning of 90s, PCVDs by late 90s, the pandemic A/H1N1 by 2009, PED in North America and Europe in 2013/14 and Senecavirus A infection in 2015 in Brazil and USA, implies to have new disease emergences every 7-8 years. Therefore, the risk of emerging and re-emerging diseases with significant economic losses and infections with unknown impact on production as well as for the human population is high and deserves preparedness and proper basic and applied research.

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DIAGNOSTIC PROTOCOLS OF INFECTIOUS DISEASES IN SWINE

G. Loris Alborali

IZSLER Istituto Zooprofilattico Sperimentale Lombardia Emilia Romagna
Animal health and diagnostic Via Bianchi7/9 25124 Brescia Italy

Introduction: Diagnostic protocols of infectious diseases in swine are important to define what an appropriate intervention strategy may be to minimize additional losses, to prevent the introduction of pathogens in a new host population, to control zoonotic pathogens and to optimize antimicrobial use. Keeping pigs healthy and productive is a major goal for producers and researchers. Preventing disease, using biosecurity and planned vaccinations, are hallmarks of well-managed swine facilities. In the case of disease outbreaks, diagnostic laboratories use an array of tests to quickly identify underlying infections and causative pathogens. Once diagnosed veterinarians can prescribe therapies to treat the infections and propose vaccinations to prevent disease outbreaks in other pigs. A comprehensive approach for the diagnosis of infectious diseases in swine should be necessary include the collection of anamnestic data, vaccination and antibiotic treatment protocol, clinical signs and timing. This approach is important also to select pathologic materials to submit for laboratory investigation. The aim of this presentation is to discuss about the diagnostic protocol used in laboratory and applied to outbreaks or surveillance programs of diseases.

Diagnostic protocols in outbreaks
Protocols can be applied to identify many diseases affecting pigs in different production phases. Diagnostic protocols for reproductive disorders in sows, respiratory diseases in post weaning and fattening pigs, enteric diseases in piglets, weaned and fattening pigs will be considered.
Diagnostic protocol of reproductive disorders in sows.
A large list of infectious agents should be included among the causes of swine abortion causing immunological deficiency, systemic infection or only locally to the reproductive organs associated with fetal death due to their direct attack to the fetus and/or the placenta. The etiological diagnosis is performed by direct (cultural, PCR, RT-PCR) or indirect (ELISA) evidence. This second method, despite its limitations (the fetal immunological competence develops in the late pregnancy), can support or even replace the first for some agents. Aborted fetuses are submitted to a complete necropsy according as previously described. If available, fresh stillborn and weak piglets are more useful than decomposing aborted fetuses. Samples of lung, heart, liver, and thoracic fluid from each fetus are collected. Organs are pooled, homogenized, and then analyzed for viral detection. In particular, PRRSV and PCV2 are detected by using RT -PCR. Aujeszky's Disease virus (ADV), Porcineparvovirus (PPV), Enteroviruses (PEV), Classical Swine Fever Virus (CSFV), and Encephalomyocarditis virus (EMCV) are detected by cultural infection cell lines and PCR. Leptosira spp is detected by RT-PCR In addition, ADV and PPV are indirectly detected by serological analysis on fetal thoracic fluid by a competitive ELISA. Bacteriological isolation and identification are performed on brain, lung, and liver tissues from all sampled fetuses using cultural method on solid agar plates in order to detect *Escherichia coli*, *Streptococcus spp.*, *Staphylococcus sp., Erysipelothrix rhusiopathiae* and *Brucella suis*.

Diagnostic protocol of respiratory diseases
Diagnostic protocol apply for respiratory diseases includes anatomo-pathological, bacteriological, Mycoplasma and virological investigations. Carcasses, lungs, heart and sometimes thoracic/pericardial fluid are submitted to a necropsy. Anatomo-pathological investigation is performed according standard practices. Lung lesions are described as acute or chronic bronchopneumonia, interstitial pneumonia, pleurisy-pneumonia, pleurisy, pericarditis and polyserositis. Samples of lung, heart, thoracic/pericardial fluid, trachea bronchial swabs from each pig were collected. Organs are used for bacteriological isolation, homogenized and then analyzed for viral and Mycoplasma detection. PRRSV, SIV and PCV2 are detected by RT –PCR and than by using cultural infection cell lines. *Mycoplasma hyopneumoniae* and *M. hyorinis* are detected by RT-PCR and, in addition, for positive samples by medium culture. Bacteriological isolation and identification are performed using generic or specific cultural method on solid agar plates in order to detect *Pasteurella multocida, Bordetella bronchiseptica , Streptococcus spp., Actinobacillus pleuropneumoniae, Haemophilus parasuis.*

Diagnostic protocol of enteric diseases
Diagnostic protocol for enteric diseases includes anatomo-pathological, parasitological, bacteriological and virological investigations. Carcasses, gastro-intestinal tract are submitted to a necropsy.

Anatomo-pathological investigation is performed according as normal practices and gastro-intestinal lesions are classified as gastritis, gastric ulcer, gastroenteritis, catarrhal enteritis, hemorrhagic enteritis, diphtheroid enteritis, colitis, chronic enteritis, proliferative enteritis, with and without lymph- adenitis. Samples of lymph-node and intestinal contents from each pig are collected and used for bacteriological isolation, for parasitological and viral detection. Coronavirus and Rotavirus are detected by RT-PCR, ELISA or electron microscopy. Isospora suis, Ascaris suum and Trichuris suis are detected by standard parasitological technique (after concentration). *E. coli* (ETEC, EPEC, STEC), Salmonella spp., *Clostridium perfringens* and *C. difficile* are isolated using generic cultural method on solid agar plates or specific enrichment selective agar medium. Detection of virulence genes of *E. coli* is performed by PCR technique using previously reported primer. Typing of Salmonella spp., Clostridium spp. is performed using PCR, serological and biochemical tests. *Brachyspira hyodysenteriae* and *B.pilosicoli* are detected using specific cultural method and RT- PCR combined. *Lawsonia intracellularis* is detected by RT PCR.

Diagnostic protocols and surveillance

The surveillance of contagious animal diseases posing a major threat to the livestock sector as Food Mouth Disease (FMD), Classical Swine Fever (CSF) and African Swine Fever (ASF) is based on global and national control strategies including laboratory contingency planning . The combination of rapid intervention and the diagnosis using tests to quickly identify infections could improve current countermeasure programs. The diagnostic protocols can be applied to the surveillance of high economic impact and emerging disease in swine farms as PRRS, SIV and PCV2. PCR and serological approach of multiple agents in serum and oral fluid samples is an important tool for diagnosis and surveillance of dynamic of infections and efficacy of vaccination programs. Scientists measure infectious disease exposure and vaccine efficacy by quantitating IgG levels and PRRSV in serum or mucosal secretions, even if the more relevant test is whether infected or vaccinated pigs produce neutralizing antibody against the virus. We know for PRRSV infections that there is a well-characterized antibody response as measured by the ELISA. As well as the absence of viremic piglets at weaning is an important indication to obtain the stabilization of a sow population. The diagnostic protocols are important for the development of a surveillance system to monitor the genetic variability and molecular epidemiology of swine bacterial pathogens as *Brachyspira hyodysenteriae* and *M. hyopneumoniae*. Genotyping information can be used in several ways: to identify prevalent strains causing disease in specific herds, to select strains to be included in autogenous or universal vaccines, to identify new virulent strains introduced into the herd, and to track potential sources of these virulent strains. Surveillance system created for swine bacterial pathogens would be an important tool that veterinarians and producers can use directly for disease control programs. Diagnostic protocol applied to detect bacterial pathogens allows to improve the monitoring of antimicrobial resistance in farms in order to identify multi resistant strains. A rapid detection of pathogens in outbreaks and the knowledge of antimicrobial resistance profile is important to use of alternative products such as autogenous vaccines and pre- or probiotics.

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Prof. Anna Oevermann

A pathogen's journey to the brain

Anna Oevermann is professor of veterinary neuropathology at the University of Bern (Switzerland). She studied veterinary medicine at the Justus-Liebig University, Giessen (Germany) and the École Vétérinaire de Nantes (France). Anna Oevermann obtained her DVM at the University of Zürich (Switzerland) and worked as a practitioner in a small animal practice in Pistoia (Italy) before performing a residency in veterinary pathology at the Institute of Animal Pathology, University of Bern. After obtaining the Dipl. ECVP in 2006 she joined the Division of Neurological Sciences at the University of Bern to specialize in neuropathology. Anna Oevermann teaches veterinary neuropathology to under- and postgraduates and is active in diagnostic neuropathology. She studies neurological diseases in animals with a focus on neuroinfectious diseases. The main research interests of her group are host-pathogen interactions and the pathogenesis of neurolisteriosis in ruminants (http://www.ekf.vetsuisse.unibe.ch/forschung/neurolisteriosis_group_anna_oevermann/index_ger.html).

A PATHOGEN'S JOURNEY TO THE BRAIN

A. Oevermann*

* Division of Neurological Sciences, DCR-VPH, Vetsuisse Faculty, University of Berne, Switzerland

Neuroinvasion by pathogens is a life-threatening event associated with high fatality rates and severe neurological sequelae because of the poor regenerative potential of the central nervous system (CNS). The CNS is protected against pathogen invasion by several barriers including the blood-brain barrier (BBB), the blood-cerebrospinal fluid barrier (BCSFB) and by the sentinel and antimicrobial activity of resident cells. Nevertheless, various pathogens (viruses, bacteria, prions, fungi, and parasites) have evolved mechanisms to penetrate the brain via different routes. Following hematogenous spread, extracellular pathogens may invade the brain by transcellular or paracellular crossing of the BBB and BCSFB. Intracellular pathogens may exploit infiltrating leukocytes to enter the brain from the blood ("Trojan horse mechanism"). The neural route of brain invasion via olfactory and trigeminal nerves, which provide direct pathways from the nose and oral cavity to the brain, but also other peripheral nerves is increasingly recognized. Additionally, pathogens may locally spread to the brain from contiguous foci of infection in the vicinity (e.g. middle ear, sinuses). In this talk, routes and molecular mechanisms of microbial CNS penetration will be discussed. Understanding pathways and host-pathogen interactions involved in CNS invasion by pathogens will advance the knowledge on the pathogenesis of CNS infections and the development of alternative treatment strategies.

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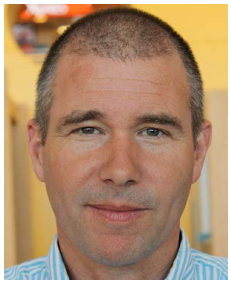

Prof. Thijs Kuiken

Emerging viral diseases from birds and bats

After graduating as a veterinarian from Utrecht University in 1988, Dr Kuiken worked in London, England, for three years as a marine mammals stranding coordinator for England and Wales. There he discovered that a large proportion of small cetacean strandings on the British coast were caused by accidental entrapment in fishing nets. He did his Ph.D. from 1993 to 1998 at the University of Saskatchewan, Saskatoon, Canada, where he characterized the epidemiology of Newcastle disease in the double-crested cormorant. Following his Ph.D., Dr Kuiken specialized in pathology and moved to the Department of Viroscience at the Erasmus University Medical Centre in Rotterdam, The Netherlands, in 1999, where he is now Professor of Comparative Pathology. He was part of the team that identified the etiological agent of Severe Acute Respiratory Syndrome (SARS), and determined that avian H5N1 influenza virus was highly virulent for cats and other carnivores. Recent achievements of his group include the discovery of novel routes of entry of influenza virus in mammalian hosts, identification of an unusual pattern of attachment of the recently emerged H7N9 influenza virus to the human respiratory tract, and elucidation of the clinical effect of influenza in the wild bird reservoir. His current focus is the pathogenesis of influenza-associated pneumonia and encephalitis, comparison of viral infections between bats and people, and identification of underlying factors for viruses to cross the species barrier from wildlife reservoirs to humans.

Prof. Louis De Tolla

The development of vaccines for emerging pathogens

Louis DeTolla, Jr. VMD, MS, PhD, Professor of Pathology, Medicine and Epidemiology & Public Health at the University of Maryland School of Medicine (UM SOM) and Founding Director of the Program of Comparative Medicine.
Dr. DeTolla regularly collaborates with a wide range of investigators who use animal models, especially in the area of infectious diseases, transgenics, biohazards, biodefense, primatology and vaccine development. His work is funded by the National Institutes of Health, the Department of Defense and industry entities. He oversees the expenditures of over 5 million dollars annually in NIH and other federal research funding.
He received a BA from Temple University in 1970, an MS in microbiology from Rutgers University in 1974, and a PhD in pathology and immunology, also from Rutgers, in 1978. In 1982, he received his VMD from the University of Pennsylvania School of Veterinary Medicine. Dr. DeTolla began his career as a research veterinarian and immunochemist at Sloan-Kettering Institute for Cancer Research. He went from there to the Fox Chase Cancer Center, where he was a research and attending veterinarian, and a cancer biologist and Chair of the Animal Care Committee in the Division of Clinical Research, and then to the Merck Institute for Therapeutic Research, where he was a research veterinarian and co-director of laboratory animal resources at the Sharp & Dohme Research Laboratories. In 1988, he joined the UM SOM in the positions he still holds today.
He has over 100 publications in areas including infectious diseases, pathology, pathogenesis and vaccine research and he is a reviewer for multiple journals including Laboratory Animal Medicine from AALAS, Comparative Medicine, Journal of Human Virology, Cancer Chemotherapy and Pharmacology, Journal of Virology and Infectious Diseases and Senior Editor of Journal of Infectious Diseases and Immunology.
Dr. DeTolla is also a longtime member of numerous professional associations, including the American College of Laboratory Animal Medicine, the American Society of Laboratory Animal Practitioners and the European Society of Veterinary Pathology. He is a member of the Board of Directors of the National Association for Biomedical Research (NABR).

EMERGING VIRAL DISEASES FROM BIRDS AND BATS

Thijs Kuiken

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In recent decades, birds and bats have been the source of important emerging viral diseases, including avian influenza viruses of the subtypes H5 and H7, West Nile virus, Severe Acute Respiratory Syndrome and Middle East Respiratory Syndrome coronaviruses, and bat lyssaviruses. I will discuss some of these viruses, comparing pathology and pathogenesis in old and new hosts, and discussing the trade-off mechanisms that determine the level of virulence. This evolutionary perspective may be useful for pathologists to understand why some pathogens cause no clinical signs whatsoever, while others nearly always kill their host.

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THE DEVELOPMENT OF VACCINES FOR EMERGING PATHOGENS

Louis De Tolla, V.M.D., Ph.D.

There are a significant number of new and very dangerous infectious pathogens that can have a highly destructive impact on both human and animal health. A few examples of these agents and ones with pandemic potential include Ebola, Lassa Fever, Rift Valley Fever, Nipah-Hendra, MERS, SARS, High Pathogenicity Influenza, Chikungunya, and perhaps Zika Virus.

At The University of Maryland School of Medicine, we have been developing vaccines for infectious diseases over the last 40 years and carry out basic research *in vitro,* in animal models, preclinical research using animal models, clinical trials in people and/or animals and GMP manufacturing of vaccines.

Today's presentation will review our University's current development of vaccines to high pathogenicity influenza, Ebola/Marburg and MERS/SARS.

Complex interactions between the three factors of virus, host, and environment determine the outcome of infection. The virus's receptors, tropism, its ability and speed of replication, mutability, surface coat stability and capacity to subvert host defenses represent initial factors of pathogenicity on contact with the host and/or maintenance within a population. The host's phenotype including innate, and acquired immunity, health, behavior and nutritional status play an important role. The third factor, the environment, plays a role as well and includes the issues of crowding, sanitation, barriers to transmission, dose of pathogen, route of exposure and inactivation of disease vectors. Vaccines, of course, seek to stimulate, enhance and specify acquired immunity which, in turn, is dependent primarily on the stimulation and activation of the T and B lymphocyte compartments of the immune system.

We investigated the efficacy of an H5N1 (influenza) DNA vaccine in a chicken and mouse animal model of vaccination and challenge. Genetic vaccines, whether DNA, RNA replicons, recombinant adenovirus etc., are of interest since they can avoid the biosafety issue of attenuated vaccines, focus on specific immunogens and avoid potentially harmful antigens, have high level of quality assurance producing the same exact vaccine in each batch, elicit T and B cell responses, combine a series of unrelated pathogens and especially allow speed and versatility in production.

The pathogenesis of influenza involves a short incubation period of 1 – 4 days and is very efficiently spread from respiratory tract secretions by the way of droplets, airborne droplet nuclei and direct contact. Shedding of the virus may occur one day prior to the development of signs/symptoms and lasts 4 – 7 days or more beyond initial symptom development. These are not insurmountable issues for a vaccine to overcome, however, the high mutation rates (antigenic drift) and mixing of genetic material (antigenic shift) make it very difficult to produce a highly effective/protective vaccine. A potential danger exists that a currently highly lethal H5N1 strain of avian influenza (Influenza A type) will mutate to allow for transmission among humans. As a result, our aim was to develop a genetic vaccine that expressed a range of homologous and heterologous immunogenic proteins (hemagglutin HA) representing multiple H5N1 serotypes. HA and cDNAs from diverse strains were inserted into pCMV plasmid expression vectors and the DNA plasmid used directly for immunization via Agro-Jet (needle-free), sub-cutaneous, and IM injections. Our results showed immune protection was conferred against a lethal challenge of A/Vietnam/1203/2004 in mice 68 weeks after vaccination. Mice had been immunized with 15 µg of plasmid DNA representing HAs from 10 H5N1 serotypes as well as two groups of 5 different serotypes and two monovalent HA. Both the group of 10, and one set of the two groups of 5 genotypes, were able to provide 100 percent protection (survival). One of the monovalent groups provided only 70 percent survival and the control (unimmunized) group, 0 percent survival. In another mouse study, a trivalent HA vaccine conferred full protection to H5N1 challenge, 68 weeks post vaccination. In chickens, we showed that protection was observed against heterologous strains of HPAI H5N1 after vaccination with a trivalent H5 serotype vaccine with doses as low as 5 µg DNA given twice, either by IM needle injection, or with a needle-free device.

Ebola is a virus that causes severe hemorrhagic fever in people (and non-human primates – NHPs) with a 90 percent plus mortality rate and no effective treatment. There have been a number of experimental vaccines studied in animal models and recently new vaccine technologies applied in the field in human clinical trials. Three strains, Zaire (EBOV), Sudan (SUVD) and Bundibugyo (BDBV) result in a high fatality rate, are endemic in tropical Africa and have the African green bat as a natural reservoir. A number of vaccine strategies exist including inactivated/attenuated virus, purified protective antigens, recombinant viruses, viral like particles (VLPs) and DNA vaccines. Three vaccine technologies that have demonstrated protection in animal models (NHP, mice, guinea pigs) use a live-attenuated recombinant Vesicular Stomatitis Virus (VSV),replication-defective Alphavirus, and a replication-defective Adenovirus which has been developed by The University of Maryland School of Medicine's Center for Vaccine Development. This recombinant viral vector vaccine, cAd3, expresses a glycoprotein (GP) antigen in a defective adenovirus backbone. It enters human cells, makes proteins including Ebola GP, but does not spread to new cells. Phase 1 clinical trails in Africa (Mali) with monovalent Ch Ad3-EBO-Z vaccine was well tolerated, immunogenic and identified the dosage to be used in all subsequent trials. A single dose could suffice for phase 3 efficacy trials of ring-vaccination containment needing short-term, high-level protection to interrupt transmission.

The WHO designated Middle East respiratory syndrome coronavirus (MERS- CoV) infection as a "threat to global health". There have been over 600 deaths with close to 2,000 human cases. It is primarily transmitted person to person in hospitals (nosocomial). It can be asymptomatic, cause respiratory distress and/or organ failure. The genome is similar to that of bat coronaviruses, with MERS-CoV RNA even found in an Egyptian tomb bat. Camels harbor the virus and have transient mild to moderate disease. Coronavirus attaches to cells by its spike glycoprotein that has a receptor-binding domain (RBD). Antibodies to RBD neutralize virus *in vitro* and in a BALB/c mouse model. There is a vaccine-induced pathology which may be due to antibody-dependent enhancement (ADE) of infection. A spike subunit based vaccine with an alum adjuvant produced a balanced Th1/TH2 response in a mouse model. Using a recombinant baculovirus, MERS S Nanoparticles were produced in a bioreactor and subsequently tested for neutralizing antibodies in mice. Currently a MERS-CoV Spike subunit vaccine leads to high titer neutralizing antibody in mice. Also, a newly designed MERS Spike vaccine with a Rhabdovirus backbone protects mice from *in vivo* challenge.

NOTES

Prof. Albert Osterhaus

Are we losing the pathologists?

Professor Osterhaus (DVM PhD) has been Head of the Department of Viroscience at Erasmus MC Rotterdam until July 1st 2014, is currently Professor of Wildlife Virology and Virus Discovery at Utrecht University, and Director of the Center of Infection Medicine and Zoonosis Research and Professor at the University of Veterinary Medicine Hannover. He has a long track record as a scientific researcher and Principal Investigator of numerous major scientific projects.

At Erasmus MC, Professor Osterhaus has run a diagnostic virology lab with more than 40 staff and a research Virology lab with over 150 personnel. His research programme follows a novel integrated "viroscience" concept, bringing together world-leading scientists in molecular virology, immunology, epidemiology, pathogenesis, and intervention studies on human and animal virus infections.

In Hannover and Utrecht he is currently heading two laboratories working in the field of One Health, that bring together world-leading scientists of multiple disciplines in this area.

Among the major accomplishments are the discovery of more than 50 viruses of humans and animals (e.g. in humans: influenza A H5N1 virus, human metapneumovirus, human coronaviruses, influenza viruses), elucidation of the pathogenesis of major human and animal virus infections, and development of novel intervention strategies. This has enabled health authorities like the WHO to effectively combat disease outbreaks like SARS and avian influenza. The spin-off, Viroclinics Biosciences BV, is another societally relevant success, allowing effective testing and refining of diagnostic tools and other intervention strategies.

The international recognition of Professor Osterhaus is further highlighted by his chairmanships of many international organizations, awards, prizes, guest lecture invitations, (co-)organiserships of international meetings and editorships of scientific journals.

Professor Osterhaus has acted as PhD mentor for more than 75 students and holds several key patents. He is also the author of more than 1100 papers in peer-reviewed journals, together cited more than 50,000 times, and his H index is 97. Most of all, Professor Osterhaus firmly believes that scientists have a role to play in translating their knowledge for the benefit and protection of society.

ARE WE LOSING THE PATHOLOGIST?

Prof. Ab Osterhaus DVM PhD

Director Research Center for Emerging Infections and Zoonoses (RIZ)
University of Veterinary Medicine Hannover, Germany.

Understanding of the etiology and pathogenesis of diseases of humans and animals has been crucial in developing intervention strategies. This was initiated and stimulated by early curiosity-driven doctors who carried out post-mortem examinations, attempting to understand the etiology of diseases, correlating symptoms, signs, and epidemiological features, with pathological changes. Similarly, when confronted today with outbreaks of emerging infectious diseases, initial etiological clues often come from correlating symptoms, signs, and epidemiological features with gross pathology, histo-pathology, immune-histochemical and *in situ*-hybridization data. Unprecedented medical, technological, and scientific progress helps us rapidly identify etiological agents, develop diagnostic assays, therapeutics and preventive vaccination strategies.

It is interesting to note that virtually all newly emerging infections in humans originate from the animal world. The complex relationships between the human and animal species have never ceased to evolve and have resulted in a human-animal interface that promoted cross-species transmission, emergence and eventual evolution of a plethora of novel pathogens. More recently, changes affecting the human population worldwide and their dramatic impact on the global environment have taken the effects of domestication, agriculture, urbanization, industrialization, and colonization to unprecedented levels. A complex mix of social, societal, technological and ecological changes, as well as the ability of certain viruses to adapt rapidly to a changing environment, has been at the basis of the emergence of new infectious agents. It appears paradoxical that while we have controlled and even eradicated major human and animal infections like smallpox and rinderpest, we are relentlessly confronted with newly emerging infections.

The modern pathologist faces the challenge of playing a pivotal role in the dynamic process from pathogen discovery all the way to development of effective therapeutic interventions and vaccination strategies. This is not limited to contributing state-of-the-art diagnostic skills, but also includes specialized knowledge to elucidate the pathogenesis of newly emerged diseases in new hosts, as well as the development of animal models. Therefore the modern pathologists should play a major role in the understanding and combat of emerging infectious diseases, by actively contributing their unique expertise to interdisciplinary teams. Fields of specific expertise include: human and animal syndrome surveillance; identification platforms for the discovery of human and animal pathogens; identification of animal reservoirs and animal models; pathogenesis study platforms, host range and transmissibility study platforms; and fulfillment of Koch's postulates. Most importantly, in this process they should take the lead in the development and exploit the use of state of the art pathology, molecular and immunological tools.

NOTES

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The WHO classification of canine lymphoma - why a diagnosis of (just) lymphoma is not enough.

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THE WHO CLASSIFICATION OF CANINE LYMPHOMA WHY A DIAGNOSIS OF (JUST) LYMPHOMA IS NOT ENOUGH

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Lymphoma has been classified in animals, primarily dogs, in many ways, often using adaptations of schemes developed for classification of human lymphoma. All major classifications distinguish follicular from diffuse lymphomas. Diffuse nodal lymphomas are the most common form of lymphoma in animals, and they may be of B or T cell origin. In most species, including dogs, B cell tumors account for 60-80% of all lymphomas. The majority of B cell lymphomas originate from cells present in follicular structures, and most commonly from cells present in the germinal centers of secondary (stimulated) follicles. Immunoglobulin (Ig) V segment somatic hypermutation occurs in the germinal center, in the process of affinity maturation (of immunoglobulin production). However, the processes involved in Ig V segment somatic hypermutation are known to target genes other than Ig genes. Constitutively greater rates of hypermutation probably explains the (much) greater incidence of B cell lymphomas compared to T cell lymphomas. Lymphomas may arise in lymphoid tissues or in non-lymphoid tissues such as intestinal mucosa and skin. Lymphomas often infiltrate multiple sites as a consequence of cell trafficking.

In people, major problems existed with many of the (previously used) lymphoma classification schemes, especially poor intra and inter-observer reproducibility. Additionally, none of these systems was shown to be superior in predicting survival, the major goal of all classification schemes. Furthermore, recognition of new entities rendered many of these systems obsolete. In the early 1990's, an informal group of 19 hematopathologists from Europe, the United States and Asia had a series of meetings and reached consensus on a new approach to lymphoma classification. It was decided that ***all*** available information would be assessed and possibly used to define lymphomas. The data included clinical features, tumor topography, cell morphology, immunophenotype, genetic and molecular genetic characteristics and clinical behavior. Different criteria were given variable importance in defining each of the different lymphoma subtypes. The cellular morphology was always important, and in some instances was the defining feature. Architecture or pattern was singularly important in defining follicular lymphomas. The emphasis on defining diseases based on utilization of ***all*** available information represented a new paradigm in recognizing lymphoma subtypes. And so, the Revised European American Lymphoma (REAL) classification was born. The inter and intra-observer reproducibility of this classification system was shown to be better (>85%) than for all other previous lymphoma classification schemes. Subsequently, the World Health Organization (WHO) convened more than 50 pathologists from around the world, as well as a large clinical advisory committee. This group largely adopted the REAL classification system, that, in 2001, became the new, revised ***World Health Organization (WHO) classification of tumors of hematopoietic and lymphoid tissues. This classification scheme has been widely accepted and utilized in human medicine.***

A number of publications have demonstrated the use of the WHO system in animals and this is the new approach utilized by the lymphoma study group of the American College of Veterinary Pathologists (ACVP). It is clear from this work that there are distinctive subtypes of canine and feline lymphoma that correspond to equivalent human entities as defined by the WHO lymphoma classification scheme. An important issue is that lymphoma is an encompassing term for a number of discrete and different clinical and morphological entities with quite different outcomes. Yet treatment strategies in animals often do not take this heterogeneity into account, typically treating most lymphomas with the same or similar protocols. It is likely that the WHO scheme will also become the pervasive scheme in veterinary medicine. Consequently, a generic diagnosis of lymphoma will no longer be sufficient as clinicians increasingly demand more specific information in order to better manage patients with lymphoma. Ideally, a ***specific*** WHO lymphoma type is diagnosed by a veterinary pathologist and that specific lymphoma type is considered when making decisions regarding therapy. In applying the WHO classification scheme to canine and feline lymphomas, it is apparent

that some critical assessments are ARCHITECTURAL eg. follicular lymphoma, marginal zone lymphoma, and hence confirmation CANNOT be made via cytology alone, although cytologic findings may suggest the (high) likelihood of a particular entity whose confirmation subsequently requires histopathologic assessment. **Additionally, in applying the WHO classification scheme to animal lymphomas, it is apparent that morphological assessments often require immunochemical stains to enable an accurate diagnosis. In some instances, molecular clonality determination by lymphocyte antigen receptor gene rearrangement analysis (see below) is also needed to confidently diagnose lymphoma versus lymphoid hyperplasia or inflammation.**

WHO classification of tumors of hematopoietic and lymphoid tissues

The following table lists the entities that were reproducibly recognized by non-specialist veterinary pathologists in the ACVP lymphoma study group.

B cell neoplasms	**T cell neoplasms**
Diffuse large B cell lymphoma - centroblastic, immunoblastic, anaplastic variants	Lymphoblastic T cell lymphoma
T cell / histiocyte rich large B cell lymphoma	Peripheral T cell lymphoma (unspecified)
Marginal zone lymphoma	T-zone lymphoma
Follicular lymphoma	Anaplastic large T cell lymphoma
Mantle cell lymphoma	Enteropathy associated T cell lymphoma
Lymphoblastic B cell lymphoma	Mycosis fungoides / Sezary syndrome
Extramedullary plasmacytoma	Hepatosplenic T cell lymphoma
Multiple myeloma	Subcutaneous panniculitis-like T cell lymphoma
Burkitt-like lymphoma	
Lymphomatoid granulomatosis (controversial)	

As stated above, while few WHO lymphoma types can be diagnosed definitively with the use of cytology alone, cytologic findings can be highly suggestive for several of the entities. This is indicated below where appropriate.

Diffuse large B cell lymphoma (DLBCL) is the most common form of lymphoma in dogs. DLBCL typically arises in lymph nodes from centroblasts in the dark zone of the germinal center. The lesion rapidly effaces the cortical architecture of the lymph node(s). Splenic involvement is also common. *Neoplastic lymphocytes are large with nuclei > 2-4 red blood cells in diameter, large prominent nucleoli and a variable, often large, volume of deep blue cytoplasm.* Three ***major*** cytologic variants are recognized – **centroblastic** with neoplastic lymphocytes having multiple large prominent peripheralized nucleoli (90% of cases), **immunoblastic, with >90% of neoplastic lymphocytes having a single, large, central, prominent** nucleolus and **anaplastic**. The cytologic appearance of these types is quite distinctive and cytologic assessment can be very suggestive of this lymphoma type. However, at a minimum, adjunctive immunophenotyping is required and architectural assessment excludes other, more uncommon, possibilities. DLBCL is a high grade lymphoma with a high proliferative fraction; it is treated with aggressive chemotherapy.
T cell and histiocyte rich B cell lymphoma (TCRBCL) is a another variant of DLBCL in which the bulk of the tumor is composed of reactive T cells and histiocytes; the neoplastic B cells are usually

<10% of the cellular infiltrate. T cell / histiocyte rich BCL is most recognized in the horse where it usually occurs as single or multiple masses in the skin and subcutis. The lesions can wax and wane for years and may terminally progress to more aggressive DLBCL with dissemination beyond the skin. ***TCRBCL is uncommon in dogs and therefore poorly characterized at this time. As the lymphoma consists mostly of small, mature appearing lymphocytes with lower numbers of admixed large immature lymphocytes and other leukocytes, the diagnosis cannot be confirmed with cytology or histopathology alone. However, TCRBCL can be one of the differential diagnoses based on routine morphologic assessment, and confirmed with immunophenotyping and molecular clonality assessments. The immunophenotyping and molecular clonality assessments can be done on remaining cytologic smears / material or done on formalin fixed, paraffin embedded material if histopathologic biopsy is done subsequent to cytology.***

Marginal zone lymphoma (MZL) is a B cell lymphoma, which may involve lymph nodes, spleen or extranodal sites (gastrointestinal tract and respiratory tract). In the spleen, MZL may infiltrate the white pulp diffusely and / or it can lead to mass formation as a solitary lesion, presenting as a large splenic nodule (most common). Different morphologic cell types may be seen in **MZL** but the most common has nuclei approximately 2 red cells in diameter with a high nucleus to cytoplasmic ratio, single, large, central nucleoli and a continuous rim of cytoplasm. **Despite this morphology, MZL is an indolent lymphoma with a low proliferative fraction (few mitoses); these lymphomas are not suited to high grade lymphoma chemotherapy protocols.** On cytology, splenic **MZL** should be suspected when this morphology is present along with few to no mitotic figures and in conjunction with the presence of a splenic nodule. However, architectural assessment and hence histopathology is required to **definitively** confirm the diagnosis of **MZL**. Cytologic assessment of nodal **MZL** often gives the impression of more aggressive forms of lymphoma and cannot reliably distinguish **MZL** from other types of lymphoma such as **DLBCL**.

Follicular lymphoma (FL) is a B cell lymphoma that may arise in lymph nodes, spleen or extranodal tissues. FL typically arises from centrocytes in the light zone of the germinal center. The follicles are enlarged and lack mantle zones. They compress the surrounding paracortical tissue. FL is characterized by a low proliferative fraction and evidence of apoptosis, which is so prominent in normal germinal centers, is lacking. Architectural assessment and hence histopathology is required to make the diagnosis of **FL**. **FL is an indolent lymphoma with a low proliferative fraction; most FL are not suited to high-grade lymphoma chemotherapy protocols.** In human medicine, FLs are graded according to the frequency of centroblasts in the neoplastic population; this has prognostic relevance especially for the highest grade (III), which is treated more aggressively. FL is rare in animals (including dogs and cats), so we do not have data on the relevance of grading FL according to the human scheme.

Mantle cell lymphoma (MCL) is a B cell lymphoma that may arise in lymph nodes, spleen, bone marrow or extranodal sites (typically the intestine). In dogs, MCL has only been seen in the spleen as a solitary splenic mass. MCL is less common than the other follicle associated lymphomas of dogs (such as MZL). MCL arises from B cells of the inner mantle zone. **Differentiation of MCL from splenic nodular lymphoid follicular hyperplasia in dogs is difficult, since the latter often consists largely of mantle cell expansion**. **Molecular clonality assessment is very useful in helping to make the distinction.** MCL is believed to be an indolent lymphoma with a low proliferative fraction. However due to the small number of cases identified to date, the true behavior is not known. In humans, MCL is an incurable disease with a median survival of 3-5 years. Adverse prognostic indicators are high mitotic rate (10 - 30/10 40x fields), peripheral blood involvement, and blastoid cytology.

Extramedullary plasmacytomas (EMP) occur commonly in skin (digits and ears) and mucous membranes of the gastrointestinal tract (oral cavity and colorectal region). The cytology of EMPs are quite diverse, which may lead to difficulty in diagnosis. Overall EMPs are benign tumors with a low proliferative fraction (mean Ki67 labeling approx. 7-12% - see below). Malignant variants occur and may metastasize widely (incidence less than 10%).
Multiple myeloma (MM) is a malignant plasma cell neoplasm that usually occurs in the medullary cavity of bones. Long lived (years) plasma cells traffic preferentially to bone marrow (via CXCR-4 and

SDF-1 / CXCL12 interactions). In this location, malignant transformation of plasma cells is known as multiple myeloma. Multiple myeloma is often recognizable as focal lucencies (bone lysis) in radiographs of the skeleton and may cause hypercalcemia. These cells often make a homogeneous immunoglobulin, resulting in a monoclonal gammopathy (recognized by serum electrophoresis).

Burkitt-like lymphoma (BLL) is a high grade B cell lymphoma with a very high proliferative fraction (approaching 100%). *The cells are intermediate in size with nuclei approximately 1.5 to 2 rbcs in diameter, round non-cleaved nuclei and multiple, prominent peripheralized nucleoli. There is typically little anisocytosis and anisokaryosis, with the characteristic uniform or homogeneous morphology giving the impression of "peas in a pod".* In people it is associated with Epstein-Barr virus (EBV) infection and consistent c-Myc translocation. This type of high grade B cell lymphoma in dogs may be associated with (even) more aggressive biological behavior and shorter survival times. The frequency of **BLL** in dogs and cats is controversial, partly because the diagnostic criteria necessary to make the diagnosis in animals need to be more clearly defined and more consistently applied.

Lymphomatoid granulomatosis (LYG) is an angiocentric, angiodestructive disease of non-lymphoid tissues (typically lung, skin, kidney and other sites). Most cases present with pulmonary nodules. In people, LYG is most often an Epstein Barr Virus+ large B cell lymphoma with an inflammatory component consisting of variable numbers of T cells, histiocytes and eosinophils. However, it may also be a high grade T cell lymphoma with much concurrent inflammation (inflamed T cell lymphoma). This is a rare disease in both humans and animals. The terminology is outdated. ***LYG*** actually refers to a pattern of disease that may be seen with several different types of lymphoma and inflammatory diseases. LYG should be viewed (most often) as a pleocellular (inflamed), high-grade large BCL or TCL with angioinvasive architecture. In people, progression to **DLBCL** occurs. Rarely, lesions with angioinvasive architecture and clonal B cell proliferation in an inflamed background have been seen in dogs and cats; these lesions have been called ***LYG***, but they should classified more specifically as an inflamed variant of large B cell lymphoma with angiocentricity. Cytologically, this type of lymphoma consists of variable numbers of large immature lymphocytes admixed with varying numbers of inflammatory cells. Confirmation of lymphoma requires immunophenotyping and molecular clonality assessments. Specific confirmation of ***LYG*** requires demonstration of an angiocentric and angioinvasive lesion, and hence requires architectural assessment (histopathology).

Lymphoblastic lymphoma (LBL) is a high-grade lymphoma of lymph nodes, spleen and occasionally extranodal tissues. In many instances there is mediastinal involvement (thymus or lymph node). It is most frequently of T cell origin (B cell LBL is less common). *Neoplastic lymphocytes in LBL have a characteristic morphology – they have intermediate sized nuclei approximately 1.5 to 2 red blood cells in diameter with fine, dense, dispersed, immature chromatin, inapparent or inconspicuous nucleoli and fine nuclear membrane irregularity.* **This morphology is actually easier to appreciate in cytologic preparations versus histopathology.** Clinically, **T-LBL** frequently present with hypercalcemia. **T-LBL** is a high-grade, aggressive lymphoma.

Peripheral T cell lymphoma not otherwise specified (PTCL-NOS) is (usually) a high-grade T cell lymphoma of lymph nodes, spleen and extranodal tissue (e.g. dermis and subcutis in skin). PTCL is a relatively heterogeneous disease and specific subtypes will likely be defined in the future. When veterinary oncologists refer to the poor prognosis of T cell lymphomas in dogs, high grade **PTCL NOS** and **T-LBL** are the diseases that justify their remarks. Nuclei are typically large, >2-3 rbcs in diameter with moderate to high N/C ratios, frequent nuclear membrane convolution and irregularity, moderate to marked anisokaryosis and variable volumes of cytoplasm. Immunophenotyping is necessary to confirm the T-cell lineage. Canine non epitheliotropic cutaneous T cell lymphoma (NE-CTCL) can have marked associated inflammation (inflamed PTCL) and is a type of PTCL that can be very difficult to distinguish from cutaneous reactive histiocytosis. Accurate diagnosis requires careful immunophenotyping and T cell molecular clonality assessment.

T-zone lymphoma (TZL) is an indolent or low grade variant of PTCL, which occurs in lymph nodes. Single (most common) or multiple lymph nodes may be involved, typically in the head region, or the disease may manifest with generalized lymphadenopathy (less common). In some instances,

the dogs present with secondary leukemia / blood involvement, despite this type of lymphoma having an indolent course (low grade). Neoplastic T cells expand the paracortex of lymph nodes, compressing the lymphoid follicles against the lymph node capsule. TZL needs to be distinguished from high grade **PTCL**, which has a greater proliferative fraction, more cellular atypia and more aggressive biologic behavior. *The neoplastic lymphocytes of **TZL** have nuclei approximately 1.25-1.5 rbc diameters, variably clumped chromatin, inapparent or small nucleoli and a low to moderate volume of cytoplasm (that stains pale blue in cytologic preparations). Cytoplasm is sometimes present as a unipolar tail, so called "hand mirror" morphology.* Cyto morphology can be highly suggestive of **TZL**. Because the neoplastic lymphocytes are small and mature appearing, cytologic confirmation of lymphoma often requires T cell molecular clonality assessment, especially when the node is not totally effaced. Additionally, the neoplastic T cells in canine TZL have a unique immunophenotype, frequently lacking CD45 expression (the leukocyte common antigen) and concurrently having weak CD21 expression (an antigen more often and more strongly expressed on B cells).

Anaplastic large T cell lymphoma (ALTCL) is a rare nodal lymphoma in which there are neoplastic lymphocytes with abundant cytoplasm and large, irregular nuclei with bizarre profiles (so called "hallmark" cells). The frequency of hallmark cells varies, but their presence is ESSENTIAL for the diagnosis. Neoplastic cells are usually cytotoxic T cells, which express CD30 and contain cytotoxic granule associated proteins such as perforin and granzyme B. In humans many cases express the anaplastic large cell lymphoma kinase protein (ALK). Expression of ALK and CD30 have not been assessed in dogs in which this disease has been observed.

Enteropathy associated T cell lymphoma (EATCL) is heterogeneous group of lymphomas that most often affect the small intestine. This complex has a high incidence in older cats; the same diseases occur in dogs at lower incidence. In cats, there is often a preceding history of inflammatory bowel disease (IBD) and lymphoma can coexist with IBD, complicating the diagnosis. Small cell EATCL, equivalent to WHO EATCL type II, is an indolent disease requiring little more treatment than IBD (e.g. chlorambucil and prednisone). *Cytologically, small, mature appearing lymphocytes predominate.* Therefore, it can be difficult to distinguish small cell EATCL from IBD**.** This is especially true if mesenteric lymph nodes are involved and are aspirated when the lymphoma has not yet effaced the node. In this situation, small mature lymphocytes predominate but there are still admixed medium and large reactive lymphocytes as well as scattered histiocytes, eosinophils and neutrophils. **Molecular clonality assessment is very useful in helping to make the distinction and can be done on DNA extracted from either cytologic or formalin fixed, paraffin-embedded material (if there is a subsequent biopsy).** *Large cell EATCL, equivalent to WHO EATCL type I, has neoplastic lymphocytes with nuclei >2 rbcs in diameter that often, but not always, have cytoplasmic azurophilic granules that may coalesce or packet in a peri-nuclear location (granular lymphocytes).* Large cell EATCL (especially the large granular lymphocyte, or LGL, form), has a much worse prognosis with a poor response to treatment and a rapid demise (usually weeks to a few months).

Cutaneous T cell lymphoma (CTCL) is a heterogeneous group of lymphomas. Non-epitheliotropic CTCL are classified mostly as high grade PTCL (see above). Epitheliotropic CTCL, also known as mycosis fungoides (MF), initially follows a more indolent course. MF usually occurs in old dogs (mean 10 years) and often has a prior history of chronic allergic or inflammatory skin disease. Neoplastic T cells have a tropism for epidermis and adnexal epithelia, and form clusters or diffuse aggregates within epithelial structures. Therefore, architecture and histopathology are required for diagnosis - and this is NOT a cytologic diagnosis. The lesions frequently involve muco-cutaneous junctions and feet (as well as other skin sites). Interestingly, these sites are target sites for atopic dermatitis, which is considered a risk factor for later development of MF. Lesions may be confined to skin for extended periods (many months to a few years) as patches and plaques, but eventually progress to tumor stage lesions (masses) with potential for distant metastasis.

Hepatosplenic lymphoma (HSTL) is a rapidly-progressive lymphoma that usually originates in splenic red pulp, usually from gd T cells, which (usually) express CD11d. There is associated liver and bone marrow involvement. This lymphoma is associated with **diffuse splenomegaly** and peripheral cytopenias (anemia, thrombocytopenia, leukopenia). These clinical associations are in part due to frequent development of a secondary hemophagocytic syndrome. Erythrophagocytic

macrophages accompany the neoplastic T cells in the spleen, liver and marrow. Lymphoma cells infiltrate the hepatic sinusoids, and also the vascular sinuses of the bone marrow. Massive splenomegaly occurs in severe cases, and peripheral lymphadenopathy is lacking. Neoplastic T cells are usually granulated (LGLs), and frequently are erythrophagocytic. *Therefore, the presence of immature appearing granular lymphocytes, sometimes erythrophagocytic, in splenic, hepatic and / or bone marrow aspirates raises the likelihood of hepatosplenic lymphoma but architecture, demonstrating sinusoidal distribution, is required to confirm this particular lymphoma type*. The clinical and morphological features of ***HSTL*** may resemble the hemophagocytic form of histiocytic sarcoma, but immunophenotyping conclusively distinguishes these 2 diseases. Hepatosplenic lymphoma has been described in dogs and horses as a high grade lymphoma (as in people); it may also occur in cats as a less clinically aggressive disease.

Subcutaneous panniculitis-like T cell lymphoma (SPTCL) is a relatively rare high grade T cell lymphoma that infiltrates the skin panniculus with almost complete sparing of the dermis. Multiple subcutaneous nodules are usually present clinically. Neoplastic T cells surround adipocytes in a peripheral ring formation. This specific type of lymphoma is therefore an architectural, and hence histopathological, diagnosis although cytology can certainly suggest the likelihood of ***SPTCL.***

Immunophenotyping

To classify lymphomas by the WHO system as applied to dogs (and cats), it is important to have access to markers for immunophenotyping. The following table lists the markers of value for determining cell lineages in leukocytic proliferations in dogs. **These markers are suitable for use in formalin-fixed paraffin embedded tissues (FFPE) with appropriate antigen retrieval protocols.** However, they can also be used on air-dried smears and on snap frozen tissue, often times more quickly, easily and cheaply. Markers are available for the detection of B and T cells. However, markers for the unequivocal detection of Natural Killer (NK) cells in dogs and cats are not available, so the existence of NK cell lymphomas is not easily confirmed. Determination of major subsets of T cells (CD4 or CD8) and T cell receptor usage (TCRab and TCRgd), as well as dissecting the lineages of histiocytes (macrophages, interstitial type dendritic cells [DC] and Langerhans cells) in histiocytic proliferative diseases is best done in cell smears (cytology - immunocytochemistry) or snap frozen tissues, or by flow cytometry (blood, aspirates of lymph node, bone marrow etc), as formalin fixation irreversibly damages these antigens and they are not assessable in FFPE.

Leukocyte markers of diagnostic importance to lymphoma investigation detectable in FFPE	
CD3ε	Signaling component of the T cell antigen receptor. Expressed by αβ T cells and γδ T cells. Cytoplasmic expression by NK cells is also possible - especially if they are activated.
CD79a	Signaling component of the B cell antigen receptor. Expressed by all stages of B cell differentiation. Expression reduced in plasma cells.
CD20	Surface molecule expressed at all stages of B cell differentiation except for plasma cells. CD20 plays a role in regulation of B cell activation and proliferation. CD20 is not completely lineage specific and has been observed uncommonly in T cell lymphomas. Caution is advised in interpretation of diffuse cytoplasmic expression, which can occur in several cell types.
Pax5	Transcription factor essential for maintenance of B cell differentiation. Useful B cell marker.
MUM1/IRF4	Transcription factor (nuclear) essential for plasma cell differentiation. Useful plasma cell marker (plasmacytomas) but not completely specific.

CD11d	αD subunit of β2 integrin (CD18) family. Expressed by macrophages and T cells in hemopoietic environments especially splenic red pulp. Bone marrow and lymph node medullary sinus macrophages also express CD11d. CD11d is consistently expressed in diseases emanating from splenic red pulp (LGL form of chronic lymphocytic leukemia; hepatosplenic lymphoma, and hemophagocytic histiocytic sarcoma)
CD18	β subunit of the β2 integrin family of leukocyte adhesion molecules. Expressed as a heterodimer of CD11a, CD11b, CD11c or CD11d with CD18. Leukocytes express at least one form of the heterodimer. CD18 is therefore expressed on ALL leukocytes. The expression level on myeloid cells is especially high compared to normal lymphocytes. CD18 has been used as a marker of histiocytes in paraffin embedded tissue, but this is dependent upon exclusion of lymphocyte differentiation by the use of other markers (CD3 and CD79a).
CD30	CD30 is an integral membrane glycoprotein and a member of the Tumor necrosis factor receptor (TNFR) superfamily. It is not expressed by resting lymphocytes, but is expressed by mitogen activated T and B cells.
CD45	Surface molecule expressed by all leukocytes - formerly known as “leukocyte common antigen”. Antibodies to CD45 bind to the extracellular domain outside of the 3 variably spliced exons (A, B, and C).
CD45RA	Splice variant of CD45 in which the A exon is present. Expressed by B cells and naïve T cells. Not typically expressed by histiocytes.
CD90 (Thy-1)	Cell surface molecule with broad cell and tissue distribution. CD90 is expressed by interstitial-type DC, but not by Langerhans cells (LC).
c-Kit	Surface molecule and member of the receptor tyrosine kinase family (type III). Expressed by most hemopoietic progenitor cells and by mast cells. Expression level is high in high grade mast cell tumors.
E-cadherin	Adhesion molecule expressed by epithelial cells and by some leukocytes. Especially useful in cutaneous round cell tumors to identify Langerhans cells (**E-cadherin** positive), indicative of cutaneous histiocytoma.
Granzyme B	Serine protease located in the granules of cytotoxic T cells (CD8+) and NK cells. GrB is expressed at high levels in activated cells and helps mediate rapid cell death by apoptosis in target cells.
Myeloperoxidase	Myeloperoxidase (MPO) is a lysosomal protein stored in the azurophilic granules of neutrophils (and monocytes). MPO is an important marker of myeloid differentiation.
Ki-67	Cell proliferation marker (nuclear) - expressed in all phases of the cell cycle except G0 and early G1. Excellent marker for determining the growth fraction of a cell population.

Once immunophenotyping is done, it may also be necessary to run molecular clonality analyses to **confirm** lymphoma when doubt still exists. This is particularly so with small, mature cell lymphomas, inflamed (mixed cell) lymphomas and lymphomas arising in the context of hyperplasia or inflammation, and with emerging or incipient lymphomas that have not yet effaced the lymph node (and hence are “mixed” cytologically).

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NOTES

Helpful advice or disparaging critic?
Your role as a peer reviewer for manuscripts in Veterinary Pathology

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Jeff Caswell is a veterinary pathologist and professor in the Department of Pathobiology, University of Guelph, Canada. Teaching and diagnostic pathology interests include respiratory and cardiac disease, inflammatory and immune-mediated disease, and teaching applied gross pathology and pathogenesis. Research focuses on the pathogenesis of respiratory disease in animals, including dysregulation of innate immune responses and the development of bacterial pneumonia, biomarkers of susceptibility to pneumonia, and the pathogenesis of *Mycoplasma bovis* pneumonia in cattle. Jeff is the Editor-in-Chief of *Veterinary Pathology*, the journal of the American, European and Japanese Colleges of Veterinary Pathology.

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HELPFUL ADVICE OR DISPARAGING CRITIC? YOUR ROLE AS A PEER REVIEWER OF SCIENTIFIC MANUSCRIPTS FOR *VETERINARY PATHOLOGY*

Jeff Caswell and Andrea Gröne

Veterinary Pathology
With credit to: Susan A. Elmore, Toxicol Pathol, and Grant Maxie, J Vet Diag Invest

Major Goals of the Review Process
- To provide topic-specific expertise in evaluation of manuscripts
- To indicate in detail the strengths and weaknesses of the paper to help authors make revisions (or understand reasons for rejection) and help editors make an informed and correct decision
- Respond in a timely manner

Why Become a Peer Reviewer?
- Contribute to science by fostering scholarly communications
- Learning experience: sharpen your own critical thinking and writing skills
- Keep up with developments in your own field
- Career-building, early in career

How do I Become a Reviewer?
- Important roles of peer reviewers
- Define the narrow topic in which you have specific expertise
- Let the journal know you are willing

Ethics
- Anonymity
- Confidentiality
- Communication to others
- Personal use of unpublished information
- Conflict of interest or a bias?
    - Close collaborator or competitor, same institution, personal relationship
    - Delaying or rejecting manuscripts of competitors

Before You Begin the Review Process
- Can you effectively review all aspects of the paper?
- Can you complete the review in a timely manner, and do it well?
- Does English usage need improvement? Does it hinders your ability to review?
- Has the work been previously published?

Title, Keywords, Abstract
- Will interested readers find the article when searching PubMed & Google? Do these effectively reflect the manuscript as a whole?
- Is the title concise, specific and informative?
- Have the most salient keywords been used?
- Does the Abstract summarize the main points of the article in adequate detail? Specifically: Study objective or background, Study design and methods, Main results, Principal conclusions

Introduction
- Provides the reader with the necessary background
- An exhaustive review of the literature is not necessary or appropriate
- Is it coherent and readable?
- Present a rationale or argument for the study
- Objectives/hypotheses/questions: stated clearly and specifically?

Materials and Methods

- Review thoroughly to ensure validity and reproducibility of the data
- Use the ARRIVE guidelines: a friend for authors, reviewers and editors
- Confirm that animal care is adequately described. What legislation is followed? Who approved it?
- Are the number of animals and other information consistent across methods, results, tables, figures?
- Assess the study design, methodology, validation & controls, interpretation
- Sample size: number per group; number of cases. How were cases obtained? Inclusion/exclusion? Allocation to treatment groups. Blinding. Assay validation, negative and positive controls. Experimental replication? Verify that the statistical analysis is appropriate

Results

- Are the results presented clearly and concisely?
- Should the raw data be made available?
- If relevant, indicate justifiable and specific additional studies needed to fill gaps

Tables and Figures

- Review all figures for scientific content. Photo Editor will review for figure quality
- Confirm that the legends adequately describe the figures, and that items described in the legend are clearly visible in the figure
- Figures and Tables should “stand alone”, allowing the reader to understand the figure without reading the text

Usual Elements of the Discussion.

- Explain and interpret the study findings.
- A literature review is not needed or appropriate.
- Justify controversial aspects of the methodology
- Acknowledge and discuss the limitations
- Discuss plausible alternative explanations
- Do the findings address the objectives/hypotheses, as stated in the introduction?
- Describe the implications and applications of the findings

Your Critique of the Discussion

- Are the interpretations of the findings adequately justified by the data, or is there excessive speculation based on inadequate data?
- Are the conclusions stated clearly, and in a way that will be useful for readers?
- Is it well-organized, reads well, and has clear and compelling insights?

References

- Do the references adequately provide the evidence on which key statements are based? (i.e. primary evidence, not statements by others in reviews or Introductions of other papers)
- An exhaustive list of references is not required

Preparing Your Review

- What is your overall opinion of the paper? State any serious concerns.
- Does it provide new information?
- Highlight strengths and weaknesses
- What contribution will this paper make to the field?
- Reviewers do not need to edit English usage, or journal style

How to be a great reviewer

- Maintain a professional and respectful tone throughout the review.
- Be objective, constructive, and specific
- Provide feedback that improves the scientific merit of the manuscript, and the communication of that science
- Give your opinion on the strengths and weaknesses of the manuscript

Sources of Information

- Summary of this presentation (*Vet Pathology* website, Advice to Reviewers): http://vet.sagepub.com/site/misc/Index/Advice%20on%20how%20to%20review%20manuscripts%20for%20Veterinary%20Pathology-1.pdf
- SAGE how to review articles. https://us.sagepub.com/en-us/nam/how-to-review-articles
- Cantor GH et al. Veterinary pathology and peer review. Vet Pathol. 2009 Mar;46:173-5.
- ARRIVE guidelines for animal studies: https://www.nc3rs.org.uk/arrive-guidelines

NOTES

Evaluation of renal biopsies in dogs and cats

Dr Jaco van der Lugt

Jaco van der Lugt graduated from the University of Pretoria (South Africa) in 1983 and has been working as a veterinary diagnostic pathologists and/or lecturer in veterinary pathology ever since. He obtained his PhD from the University of Utrecht and is a Diplomate of the European College of Veterinary Pathology (ECVP). He joined IDEXX in 2010 as a veterinary anatomical pathologist and has a special interest in renal pathology and dermatopathology.

He is a member of the World Small Animal Veterinary Association–Renal Standardization Study Group and in this capacity was responsible (in collaboration with Dr Luca Aresu, Padova, Italy) for reporting on renal biopsies submitted to the Utrecht Veterinary Nephropathology Service. This veterinary nephropathology service began examining renal biopsies at Utrecht University, The Netherlands, in 2008 and since 2015 operates as the International Veterinary Renal Pathology Service located within the Department of Comparative Biomedicine and Food Science at the University of Padova, Italy, under the leadership of Dr Luca Aresu.

EVALUATION OF RENAL BIOPSIES IN DOGS AND CATS

JJ van der Lugt

The main aim of performing a renal biopsy is to obtain information that may assist a clinician to manage a patient's illness more astutely than might be possible without the biopsy and thus to obtain the best available outcome[5,6]. In the past, renal biopsy in dogs has been utilised infrequently by veterinarians and reasons for this may include sampling of renal biopsy not in an early stage but in an advanced stage of kidney disease whereby pathological findings may be of less or questionable clinical use; diagnostic pathologists utilising limited techniques in the examination of biopsies; and insufficient integration of pathological findings with appropriate clinical data, both by clinicians and pathologists[5,6].

This infrequent use of advanced diagnostic techniques such as immunostaining and electron microscopy and the uncertainty regarding the accuracy of a diagnosis based solely on histopathology have created concerns regarding the clinical utility of canine renal biopsy. This could in part be explained by the nonstandardized, often retrospective, analysis of renal tissue by veterinary pathologists. Furthermore, canine glomerular diseases share many of the morphological characteristics seen in their human counterparts, but there are striking differences as well.

To address these concerns, the World Small Animal Veterinary Association–Renal Standardization Study Group (RSSG) was conceived at Netherland's Utrecht University in January 2005[4]. This international group of veterinary nephrologists and pathologists set out to design a study that would develop a comprehensive understanding of glomerular disease in dogs by routinely using standardized light microscopy, immunofluorescence microscopy and transmission electron microscopy to evaluate renal biopsies and by associating the pathologic findings with detailed clinical and case outcome data. Two veterinary diagnostic renal pathology centers were established to perform the evaluations and facilitate the collection of cases for prospective studies. The United States center (created in 2005 at Texas A&M University as the Texas Veterinary Renal Pathology Service) was reorganized in 2013 as the International Veterinary Renal Pathology Service and now operates as a joint effort between The Ohio State University and Texas A&M University. The Utrecht Veterinary Nephropathology Service began examining renal biopsies at Utrecht University, The Netherlands, in 2008 and was reorganized in 2014, currently operating as the International Veterinary Renal Pathology Service (located within the Department of Comparative Biomedicine and Food Science, University of Padova, Italy).

Indications, contraindications, planning, biopsy specimen acquisition and initial assessment and processing of renal biopsy specimens are described elsewhere[5,6] and will be briefly reviewed in the lecture at the ESVP-ECVP meeting. Renal biopsies at both veterinary nephropathology services are routinely evaluated by light microscopy, immunofluorescence microscopy and transmission electron microscopy. In addition to the usual haematoxylin and eosin (H&E) staining of paraffin sections, thin histological sections are also stained with Periodic acid-Schiff which stains glomerular and tubular basement membranes, mesangial matrix and Bowman's capsule basement membrane; Jones' methenamine silver (or periodic acid methenamine silver stain, PAMS) allowing visualisation of the glomerular basement membrane; and Massons trichrome stain allowing not only evaluation of scarring but also allows visualisation of immune complexes as red granular material along the glomerular basement membrane. Other stains such as for fibrin and amyloid may be used[3].

The presence of suspected glomerular disease is the most common reason to consider performing renal biopsy in the dog. Proteinuria is the hallmark of glomerular injury and this abnormality often is a key factor that prompts consideration of a renal biopsy. Recently, the Renal Standardization Study Group published results from a study to classify glomerular disease in the dog[2]. Dogs in this study had renal biopsies performed because they exhibited proteinuria indicative of the presence of glomerular disease—that is, persistent renal proteinuria with urine protein:creatinine ratio (UPC) values ≥2.0—which was subsequently confirmed by biopsy findings. Eight veterinary pathologists evaluated 114 parameters (lesions) in renal biopsy specimens from 89 dogs. Hierarchical cluster

analysis of the data revealed 2 large categories of glomerular disease based on the presence or absence of immune complex deposition. Firstly an immune complex–mediated glomerulonephritis (ICGN) category included cases with histologic lesions of membranoproliferative or membranous patterns. The second category included control dogs and dogs with non-ICGN (glomerular amyloidosis or focal segmental glomerulosclerosis).

The Renal Standardization Study Group also formulated guidelines for the pathological evaluation of renal biopsies[1] and, in order to achieve standardisation of nomenclature in veterinary nephropathology, published a list of definitions of light microscopic, ultrastructural and immunofluorescence lesions of the kidney[2].

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NOTES

Proposal for a practical approach to alopecic biopsies

Prof. Paola Roccabianca

In 1991 graduated in Veterinary Medicine with honors at the University of Milan. In 1994 obtained her Doctorate degree in comparative pathology with a thesis on pathological and molecular characterization of canine lymphomas. In 1995 obtains a J. Fullbright scholarship spent as a post-doctoral fellow at the University of Davis, California with a project on feline mucosal immunology and feline LGL leukemia/lymphoma. In 1998 obtained a tenure track investigator position at the School of Veterinary Medicine of Padova where opens the histopathology diagnostic service and teaches immunopathology, dermatopathology, necropsy techniques and histopathology to veterinary students. In 2000 obtains the European board certification in Veterinary Pathology (ECVP diplomat). In 2004 becomes Associate professor the Unverisity of Milano. Currently is associate professor of pathology at the University of Milano and is the director of of the ECVP certified residency program in Veterinary Pathology that has diplomated over 20 Veterinary Pathologists and participated to the training of veterinary dermatologists and surgeons. Responsible of special pathology and laboratory animal pathology courses for the Veterinary Biotechnology School, and is responsible of the dermatopathology and non-conventional pet diagnostic histopathology services.

Research interests mainly focused on cutaneous small animal pathology and on the characterization of lymphoproliferative disorders in cats. In 2007 receives the CL Davis Foundation Journal Award for the best veterinary pathology paper published in 2006with the publication “Feline Large Granular Lymphocyte (LGL) Lymphoma with Secondary Leukemia: Primary Intestinal Origin with Predominance of a CD3/CD8a/a Phenotype”. Member of several committees including member of the Exam Committee of the ECVP (2003-2005), Board member of the International Society of Veterinary Dermatopathology (2004-2006), President of the ECVP Exam Committee (2006-2008), 2011-2015 ECVP Council member (councillor).

Author of over 60 publications in peer reviewed scientific journals indexed in med line (Citations: 765, h-index: 16 in Scopus)

PROPOSAL FOR A PRACTICA APPROACH TO ALOPECIC BIOPSIES

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Introduction: In the Ackerman based veterinary dermatopathology scheme a 9th "pattern" was added to designate "Endocrinopathy" comprising non-inflammatory alopecic disorders. When it became apparent that many of the follicular diseases with this "pattern" were not endocrinological the pattern was changed onto Atrophic. However, many of these diseases are not atrophic, but rather perturbations of hair cycle or abnormalities of growth and, some may represent the end-stage of inflammation. Thus, the definition of Non-Inflammatory Follicular Disorders (NIFD) seems more appropriate. The diagnosis and classification of NIFD are complex and not always straightforward for several reasons including variation in clinical presentation, marked breed variation in hair cycle. Pivotal for the diagnostic approach to NIFD are adequate biopsy samples (> 8 mm with panniculus and taken from areas with maximal alopecia), signalment and relevant clinical history and knowledge of hair follicle microanatomy.

Microanatomy of HF: There are two types of hair follicles simple (human, equine, bovine, porcine) and compound (canine, feline, ovine, caprine). In simple HF, each hair shaft is associated with one HF ostium while in compound HF a primary hair and multiple secondary hairs exit from the same ostium. The primary follicles (usually 3-5 per unit) are the largest and the most cranial. Behind these are arrayed the secondary follicles in decreasing size. HF are composed (during anagen-growth phase) of three portions, infundibulum: composed of a stratified squamous epithelium with epidermal keratinization (keratohyaline granules), extending from the orificium to the insertion of the sebaceous gland duct; Isthmus: spanning the HF portion from the insertion of the sebaceous gland to the attachment of the arrector pili muscle and characterized by inner root sheath keratinization in anagen and outer root sheath keratinization in telogen growth phases; Inferior segment extending below the insertion of the arrector pili muscle to the hair bulb. Distinction of the three sections is possible through the recognition of the keratinization, infundibulum finishes where it is possible to observe the end of trichilemmal keratinization, while the isthmus extends down to the interface between the cornified inner sheath and the first non cornified cells of Huxley's layer (top end of trichohyaline granules). Hair follicles are composed of:

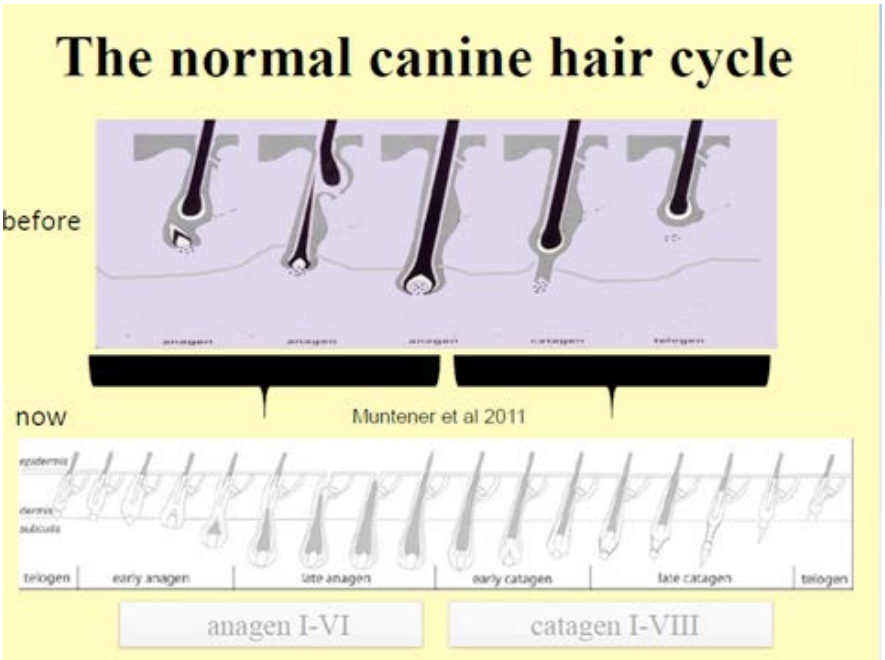
Dermal hair papilla connective tissue structure covered by a thin continuation of the basement membrane

Hair matrix the lower part of the hair bulb is composed of undifferentiated hair matrix cells. Consists of layers of epithelial cells (germinative cells) that undergo mitosis regularly. Growing hair matrix is one of the most rapidly proliferating tissues in the body. The pool of stem cells that forms the matrix of the follicle produces the elaborated follicular structure that consists in the different portions of the hair shaft (medulla, cortex and cuticle) as well as the layers of the inner root sheath.

Inner root sheath (IRS) composed of three concentric layers. The inner most is the IRS cuticle (single layer of cells that interlocks with the hair shaft cuticle). The middle layer is the Huxley layer that consists of one to three nucleated cell layers. The outermost layer is composed of a single layer of anucleated cells and is named Henle's layer. The inner root sheath can be identified histologically by the content of **trichohyaline granules** (bright red granules). This portion of the follicle keratinizes fully and disappears at the level of the *isthmus.*
The Companion cell layer (CK75+) is a single layer of flat cells in between the IRS and the ORS that originates from a separate cell lineage with a stem cell/transient amplifying mode of growth that parallels IRS mode of production. **Outer root sheath (ORS)** this portion is contiguous with the interfollicular epidermis. The ORS is composed by basal cubical cells expressing CK14 with a distinctive growth zone localized in the suprabulbar area. In the inferior segment of the follicle the outer root sheath is covered by the inner root sheath and does not keratinize. At this level the cells have clear, vacuolated cytoplasm (high glycogen content). At the *isthmus* the outer root sheath is no longer covered by the IRS and undergoes trichilemmal keratinisation. In the infundibular segment,

the outer root sheath keratinizes similarly as the surface epidermis during telogen phase while in anagen the fully cornified inner root sheath cvovers the outer root sheath.

Hair follicle cycle recognition: generally there are 4 phases in the hair cycle: 1) anagen (active growth phase), 2) catagen (relatively short regression stage, <3% catagen follicles in normal skin), 3) telogen (the resting stage) and 4) exogen (when the hair shaft is expelled). The term "kenogen" has been proposed to describe non-haired telogen follicles. These phases have been subdivided in dogs by Muntener et al, 2011.



The most important features to recognize these phases are

i) Position of the dermal papilla (DP) in dermis or subcutis
ii) Position of the DP relative to the HF
iii) Length of the hair shaft

For the accurate evaluation of HF cycle stages:

i) Serial sections are necessary
ii) Visualization of the DP with anti-laminin or anti-vimentin staining
iii) Visualization of the early stages of anagen (IRS development) with modified Sacpic technique (stains non-keratinized tissues blue with yellow granules and keratinized bright red).
iv) To differentiate anagen from early catagen Masson-Fontana silver stain identifies melanin granules.

Limitations of cell cycle stage recognition in histological sections:

a) Anatomic differences - for example mane and tail hairs in horses should always be in anagen.
b) Breed differences - length of the anagen cycle is breed associated - e.g. Poodle hair is in continuous anagen (98% hairs will be in anagen). Many breeds have a telogen predominant hair cycle in which >50% of follicles will be in telogen (Arctic breeds as an energy saving feature). This is also affected by seasonality of shedding. Using the kenogen phase seems helpful.
c) We know little about environmental effects such as climate, photoperiod on pet hair development and cycling.
d) Technical limitations – even with serial sections, 35% of follicles have been considered not classifiable by trained pathologists. Also, suggested need to count 50 follicles (not always present in biopsy samples).

Classifications: NIFD classification changes from author to author however, several entities do not fit completely into any of the attempted schemes. Gross et al. (2005) separate non inflammatory disorders of the adnexa into dysplastic diseases including NIFD with abnormal HF development and growth suspected or proven to be hereditary and into atrophic diseases comprising disorders of HF cycle growth arrest with/without atrophy. However, the same authors conclude that atrophic diseases can associate with dysplasia (intended as misshapen follicles). Mecklenburg et al. (2009) apply a more complex classification based on etiology and pathogenesis NIFD into: 1) Congenital +/- hereditary alopecia (with HF and adnexal aplasia or with HF dysplasia); 2) Alopecia due to trichomalacia; 3) Alopecia with abnormal HF cycling +/- dysplasia; 4) HF dystrophy +/- atrophy; 5) Traumatic; 6) Scarring.

Proposed approach to microscopical diagnosis: NIFD associate with alopecia as one of the major clinical signs in association with other epidermal changes. Dr. J. Yager provides with a mixed practical approach based on the identification of specific microscopic clues for the diagnosis of some of the most frequent NIFD. The following are useful clues for the approach to alopecic biopsies:

1) Lack of adequate number of HF +/- (no follicles at all, or rudimentary and/or incompletely formed), +/- dermal fibrosis, +/- faded follicles. In this group can be included X-linked ectodermal dysplasia (hypohydrotic/anhydrotic ectodermal dysplasia), canine ectodermal dysplasia, congenital alopecia in certain cat breeds, cicatricial alopecia, scarring alopecia, traction alopecia).

2) Presence of dysplastic follicles (intended as abnormal morphology of HF at any stage) associated either with pigmentary changes (color dilution alopecia & black hair follicular alopecia), excessive trichilemmal keratin (canine alopecia X, post clipping alopecia), infundibular distortion (canine recurrent flank alopecia), follicular miniaturization (canine pattern alopecia).

3) Evidence of hair cycle arrest (high Kenogen:Anagen ratio**)** with or without atrophy.
Muntener et al. (2012) showed no difference in percentage of telogen follicles between alopecic biopsies (from all causes) and biopsies from normal dogs. There was a significant increase in kenogen and a decrease in anagen (and catagen but the latter were present at a low rate) in the alopecic dogs. If one takes the data from that paper and calculates a ratio of kenogen to anagen (K:A) there is a dramatic difference between normal dogs and dogs with alopecia. K:A was higher in Alopecia X and hyperestrogenism and lower in hypothyroidism and recurrent flank alopecia In Alopecia X and hyperestrogenism a higher number of telogen HF were observedas compared to the other alopecic disorders. In this group hyperadrenocorticism, canine hypothyroidism, canine hyperestrogenism, feline paraneoplastic alopecia can be also included.

4) Follicles with normal proportion of cycle phases in the face of clinical alopecia or hypotrichosis.

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NOTES

INTERACTIVE VETERINARY NEUROLOGY-NEUROPATHOLOGY WORKSHOP

CORRELATION BETWEEN CLINICS, NEUROIMAGING AND NEUROPATHOLOGY IN CENTRAL NERVOUS SYSTEM (CNS) DISEASE: HOW CLINICIANS AND PATHOLOGISTS CAN LEARN FROM EACH OTHER

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** TiHo Hannover, University of Veterinary Medicine Hannover, Germany*
† Vetsuisse Faculty, University of Berne, Switzerland

The introduction of non-invasive neuroimaging techniques for the exploration of the diseased central nervous system (CNS) has revolutionized veterinary neurology. Over recent years, magnetic resonance imaging (MRI) has emerged as the primary non-invasive technique complementing the neurological examination. The availability of stereotactic brain biopsy devices in veterinary medicine will further promote the diagnostic workup of neurological cases. Due to the complex CNS anatomy and the complexity of diseases affecting the CNS, a good cooperation between clinicians and pathologists aiming to correlate clinical neurology, neuroimaging and neuropathology significantly furthers the diagnosis of neurological diseases. This cooperation will become increasingly important with the development and availability of specific therapies for neurological diseases. Visualization of CNS lesions by MRI helps the clinical neurologist to narrow down the list of differential diagnoses and to come to a definitive diagnosis in some cases. Knowledge on neuropathology is required for the interpretation of MRI images. On the other hand, the accurate neuroanatomical localization by the clinician aids the pathologist to focus the neuropathological evaluation. MRI is more sensitive than gross neuropathology, and many lesions are more accurately evaluated on MRI than on gross examination. In the investigation of biopsies, MRI images serves as a surrogate of gross pathology. Therefore, clinical information and MRI features should be integrated into the interpretation of neuropathological data. This workshop aims to demonstrate the benefits of correlating clinical neurology, neuroimaging and neuropathology for the diagnostic workup of CNS diseases by interactively discussing neurological cases of small and large animals.

NOTES

- **Achim D. Gruber: Science Slam "Over the Fence" – Proposal for a New Presentation Format at the Annual ESVP/ECVP Conferences**
- **Nancy Erickson: The Worm Within: A Totally New Kind of Carcinogenesis**
- **Stefanie Binder: Neutrophil Extracellular Traps: The Neutrophil´s Lethal Weapon 3.0**
- **Nancy Erickson: Pigeon Pathology: Pattern Recognition Made by Mother Nature**

The annual ESVP/ECVP congresses aim at offering top notch up to date scientific exchange and continuing professional education. Specifically, key note lectures and workshops given by experts in their fields and the science conducted by the delegates themselves have traditionally dominated the program. However, we experience novel breaking developments and knowledge with high relevance to veterinary pathology that in principle cannot all be covered by key note lectures and which are not yet part of the delegates´ scientific activities. To close this gap, we propose to add a new presentation format to the congresses that inform the delegates about such recent developments in our or adjacent fields, somewhat resembling a focused, oral type of journal club contribution. For increased palatability, such presentations could also contain a broader perspective view, more background information, some critical appraisal and interpretation for veterinary pathology and possibly even some entertaining elements. The contributions would be given as short, focused oral presentations by those delegates who have discovered the development and judge them important for our community. As several of the criteria match the typical features of "Science Slams", this recently emerged format may serve as a promising template, however, with the exception that regular science slams typically contain own work performed by the presenting person.

As a start, we propose to have 5 to 8 min oral presentations by volunteers, using a power point or comparable presentation tool with little writing but own graphical illustrations and schematics, complemented by original work by other scientists, e.g., key images or graphical elements from publications by others, usually third party authors. Importantly, sources of external information and images need to be referenced and acknowledged. Largely depending on the contributors personal preference, such a presentation may also contain some entertaining elements for ease of acceptance by the audience, similar to the typical science slam format. Naturally, this kind of presentation would not represent an original scientific contribution. For your own preparation, you may want to visit the YouTube platform to get familiar with typical science slams and their wide spectrum of possible elements.

We feel confident that the annual ESVP/ECVP meetings could largely benefit from such a novel and exciting presentation format that would add significant information on new developments to veterinary pathology, from both inside and outside of our field, that would otherwise not be included in the program. Here in Bologna 2016, we offer three such short presentations given by volunteers with great ideas. Clearly, the format proposed here as a modified Science Slam will be subject to discussion and maturation through the delegates, the Scientific Committee of the ESVP/ECVP and future organizers. We look forward to your comments and suggestions on this initiative.

NOTES

ORAL PRESENTATIONS

Session A - General Pathology

1

RNA INTERFERENCE SCREEN REVEALS DIFFERENTIAL EFFECTS OF HOST CELL FACTORS ON TRANSDUCTION BY ADENO-ASSOCIATED VIRUS 2 (AAV2) VECTORS

Francesca D. Franzoso[*], Artur Yakimovich[†] Kurt Tobler[*], Adrian Man[*], Rebecca Vogel[*], Urs Greber[†], Mathias Ackermann[*] and Cornel Fraefel[*]

[*]Institute of Virology, VetSuisse Faculty and [†]Institute of Molecular Life Sciences, of University of Zürich, Zürich, Switzerland

Introduction: AAV2 is a small, nonenveloped parvovirus, with a 5 kb long, single-stranded linear DNA genome. In coinfection with a helpervirus (typically a herpesvirus or an adenovirus), AAV2 can establish a productive infection. AAV2 has attracted considerable interest as a platform for the design of vectors for gene therapy, particularly in treating genetic disorders. Their efficient transduction in vivo is limited to postmitotic cells. We performed an siRNA screen to assess the effect of 65 host cell proteins previously identified as components of AAV2 replication compartments, on the transduction efficiency by single-stranded (rAAVGFP) and self-complementary (scAAVGFP) AAV2 vectors.

Materials and Methods: Viral transduction (GFP fluorescence) was recorded in HeLa cells by high-throughput microscopy using an ImageXpress High Content Screening System (Molecular Devices) and further analyzed with CellProfiler (BROAD Institute, Cambridge, USA) and KNIME (KNIME.COM, Zurich Switzerland) software. The post-transcriptional silencing of the target genes was confirmed and the data on gene expression validated by RT-qPCR and Western blotting.

Results: We found that knockdown of several cellular genes, such as Mre11, Nbs1 and Rad50, enhanced the transcription from both single-stranded and scAAV2 vectors, while the knockdown of other genes, such as replication proteins (RPA1 and RPA2) and DNA mismatch repair proteins (MSH2, MSH3, and MSH6), differentially affected gene expression from the two different vectors.

Conclusions: We identified a number of cellular proteins that either positively or negatively affect transduction efficiency by AAV2 vectors. These findings may have further implications for studies on AAV2 biology and for improving AAV2 vector-mediated gene therapy.

NOTES

2

NEUTROPHIL EXTRACELLULAR TRAPS (NETS) IN SELECT ANIMAL DISEASES, DETECTED BY IMMUNOHISTOCHEMISTRY

M. Fragoso, S. Binder, K. Dietert and A. D. Gruber

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Introduction: Neutrophils effectively contribute to the innate defense against bacterial infections through phagocytosis and degranulation of, e.g., bactericidal peptides. Recently, a third and completely novel class of extracellular defense mechanism has been discovered in humans, termed Neutrophil Extracellular Traps (NETs). The release of NETs, a process called NETosis, includes a unique programmed cell death pathway that results in the ejection of nuclear DNA and citrullinated histones, decorated with antimicrobial peptides including neutrophil elastase (NE) and myeloperoxidase (MO). NETosis has not yet been characterized in domestic animals.

Materials and Methods: Four groups of diseases were tested: bacterial (*n*=62), fungal (*n*=9) and parasitic (*n*=2) infections as well as coagulopathies (*n*=20) from 71 dogs and 22 horses. The archival formalin-fixed, paraffin embedded tissues were immunohistochemically studied with antibodies against citrullinated histone H3, NE and MO. Nuclear DNA was visualized using the DAPI stain.

Results: NETs were identified by co-localized signals of NE, MO, H3 and DAPI only in acute or subacute bacterial or fungal infections, primarily adjacent to the pathogens, necrotic debris or Splendore-Hoeppli material. NETosis was also visualized in thrombi in splenic infarctions and/or hematomas.

Conclusions: The methods used yielded signals consistent with the previous detection of NETosis in humans, both in terms of antibody reactivity and location of the signals. Not surprisingly, NETosis seems to also occur in dogs and horses and likely other animals. This is only the start of more comprehensive and systematic studies of this newly discovered phenomenon and its relevance for veterinary pathology.

NOTES

3

CARBON NANOTUBES: INSIGHT INTO TISSUE DETECTION METHODS AND THEIR SHORT-TERM SYSTEMIC TOXICITY

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Introduction: There is growing interest in recent years in introducing carbon-nanotubes (CNT) based materials in biomedical applications. Despite fascinating properties, the utilization of CNT raise major concerns regarding their toxic impact. Our study aims to respond to some of the urgent questions regarding the toxicity and analytical methods used for the assessment of CNT toxicokinetics following systemic exposure.

Materials and Methods: A single-dose of purified and solubilized, short, multi-walled CNT was intraperitoneally instilled into albino-rats. The CNT distribution and morphological changes associated with the nanoparticles presence in paraffin and plastic-embedded tissues were evaluate by classical histology, high-resolution dark-field microscopy coupled with hyperspectral imaging (HRDF-HI), transmission electron microscopy (TEM), confocal scanning laser microscopy (CSLM) and micro-Raman spectroscopy (MRS). Furthermore, tissue expression for Cleaved Caspase-3, alpha-smooth muscle actin and pancytokeratin was assessed by immunohistochemistry and immunofluorescence.

Results: Tissular localization of CNT is significantly improved by HRDF-HI. The use of plastic embedded semithin sections enhance the sensibility of HRDF-HI technique for nanoparticle detection, permitting their quantitative measurement in tissue sections and thereby establishing CNT toxicokinetics. Histologically, sinus histiocytosis and multifocal granulomatous peritonitis were the main changes. An interdependence among the CNT biodistribution and local expression of Cleaved Caspase-3 was observed especially in the peritoneum-associated lymphoid tissue, locally lymph nodes and liver. Our results also indicate that an important population of peritoneal myofibroblasts are the result of epithelial-mesenchymal transition from the local mesothelial cells.

Conclusions: A strong correlation between the toxicokinetics of CNT as determined by HRDF-HI and local toxic changes was observed.

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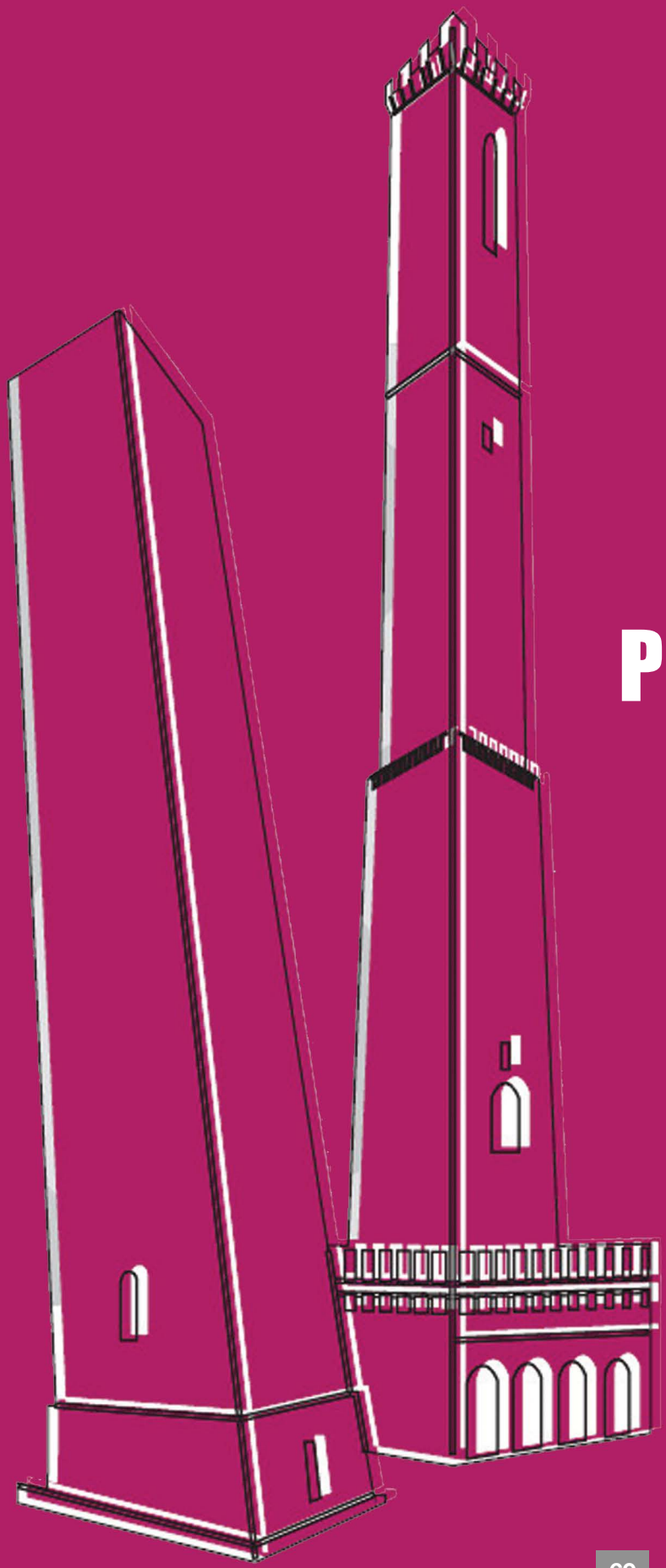

ORAL PRESENTATIONS

Session B - Fish/Wild

4

A RETROSPECTIVE REVIEW OF DISEASE IN CAPTIVE PENGUINS

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International Zoo Veterinary Group Pathology, Leeds, United Kingdom

Introduction: This report reviews diseases of 9 captive penguin species submitted to International Zoo Veterinary Group Pathology between 2003 and 2016.

Materials and Methods: Gross post mortem and histology diagnoses of 374 animals were categorised into 14 disease categories, subcategorised by cause/aetiology and affected organs. Case materials included necropsies and formalin-fixed tissues submitted from zoological, rehabilitation, or aquarium facilities. Data was analysed using pivot tables to evaluate the prevalence of disease by system, process, aetiology, and organs.

Results: Animals originated from 46 facilities and 4 referral pathology practices within the UK, Europe, the Middle East and Asia. Humboldt's penguins (n = 201, 54%) and African penguins (n = 71, 19%) were the most commonly submitted species. Disease processes included infectious/inflammatory (49%), deposition (9%), environmental and nutritional (5%), cardiovascular (4%), degenerative, developmental and haematopoietic (3%), reproductive and trauma (2%), neoplastic, toxin-associated and miscellaneous disorders (1%). No diagnoses were made in 12% of cases.

Most significant infectious disorders comprised fungal (37%), bacterial (36%) and parasitic (16%) infections. Aspergillosis (79/84, 94%), necrotising gastroenteritis (28/80, 35%) and avian malaria (33/36, 92%) were the most prevalent diseases in each category. Urate accumulation (gout) was the most significant deposition disorder (28/42, 67%). Gastric foreign bodies dominated the environmental disease category (13/19, 69%).

Conclusions: No previous similar review of captive penguin pathology data has been reported. Findings highlight the importance of addressing aspergillosis, avian malaria and gastric foreign bodies in captivity, diseases rarely seen in wild penguins. Necrotising gastroenteritis of penguin chicks is poorly understood and warrants further investigations. This is the first report indicating the prevalence of neoplasia in captive penguins (1%), a figure likely to increase as improved husbandry prolongs captive longevity.

NOTES

5

NIDOVIRUS INFECTION OF GREEN TREE PYTHONS: IDENTIFICATION AND MORPHOLOGICAL FEATURES OF A FATAL RESPIRATORY DISEASE IN *MORELIA VIRIDIS*

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Introduction: We recently observed a noticeable increase in the diagnostic submission of green tree pythons (*Morelia viridis* (SCHLEGEL, 1872)) with sudden death. All had in common an accumulation of mucus within the airways/lung associated with an apparent thickening of respiratory epithelia. Here we report on the pathological and virological findings in such cases of "mucoid pneumonia".

Materials and Methods: Full necropsy and (quantitative) histological examination was performed on 12 green tree pythons from 7 different breeders, all with gross evidence of "mucoid pneumonia". For identification of a causative agent, next-generation sequencing (NGS), virus isolation, ultrastructural examination and qRT-PCR were employed. Five lesion-free animals served as controls.

Results: The main and consistent findings were a marked hyperplasia of the respiratory epithelium, hypertrophy of the aveolar epithelium and accumulation of mucus in the distal airways. NGS and *de novo* sequence assembly detected a partial nidovirus genome with <85% nucleotide identity to nidoviruses previously identified in other pythons. A qRT-PCR covering all known python nidoviruses demonstrated nidovirus RNA in the lungs of all affected animals. Also, viruses with cytopathic effects and viral particles morphologically consistent with Nidovirus were isolated in tissue culture.

Conclusions: Nidovirales, several of which cause respiratory disease in other species, appear to represent an emerging causative agent of fatal pneumonia in several python species. Investigations into the pathogenesis of the infection are now needed, and our findings indicate that python nidoviruses can be both cytopathic and proliferative for the respiratory tract epithelium.

NOTES

6

ROLE OF *VIBRIO TAPETIS* IN THE DEVELOPMENT OF SKIN ULCERATION IN COMMON DAB (*LIMANDA LIMANDA*)

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Introduction: An increasing prevalence of skin ulcers in flatfish in the North Sea has been noticed. The cause is hitherto not known. However, we have isolated *Vibrio tapetis* as a pure culture from several lesions. The aim of this study was to elucidate the role of *V. tapetis* in the development of skin ulcers.

Materials and Methods: On the pigmented and non-pigmented skin of 60 individually tagged wild-caught dab three adjacent areas were demarcated: a zone where skin was descaled; a zone where mucus was removed and an intact zone. The order of the three treatments was randomized for all individuals. Immediately thereafter, a group of 36 dab was challenged with *V. tapetis* by immersion (3.28×10^5 colony forming units/ml) during one hour. The other 24 animals were sham treated (controls). Fish were daily monitored for clinical signs and gross lesions during 21 days. In animals that died or have been euthanized, gross lesions were scored. At necropsy, samples were taken for histological, immunohistochemical and bacteriological examination.

Results: In the challenged group, significantly more animals died compared to the controls. Lesions were significantly most severe in descaled areas and in challenged dab. Preliminary results of bacteriology and immunohistochemistry confirmed the presence of *V. tapetis* in the lesions.

Conclusions: Our preliminary results point towards *V. tapetis* as possibly having an etiological role in the development of skin ulcerations in dab subsequent to skin damage. However, this research hypothesis needs to be verified by on-going analyses.

NOTES

7

ORGAN DISTRIBUTION OF THE NOVEL ZOONOTIC VARIEGATED SQUIRREL 1 BORNAVIRUS (VSBV-1) IN NATURALLY INFECTED SQUIRRELS

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Introduction: Recently, a novel zoonotic bornavirus was detected in a variegated squirrel (*Sciurus variegatoides)* which was associated with a fatal encephalitis in three squirrel breeds. VSBV-1 infection in humans is strictly neurotropic, whereas in squirrels a widespread organ distribution was found.

Materials & Methods: Gross pathology, histopathology, immunohistochemistry and a pan-bornavirus RT-q PCR for the presence of viral antigen and RNA was carried out in six variegated squirrels and three Prevost´s squirrels (*S. prevostii*). Samples from central and peripheral nervous system, respiratory, gastrointestinal, urogenital tract, skin and lymphatic tissues were tested.

Results: Histopathology revealed no or mild non purulent meningitis/encephalitis in some of the squirrels and intranuclear inclusion bodies. Viral antigen was detected in nervous tissue (brain, spinal cord, peripheral nerves and ganglia) and in respiratory, gastrointestinal, urogenital tract and skin. High genome loads were detected in the brain, kidney and skin. This indicates a broad cell and organ tropism of the virus not only restricted to nervous tissues.

Conclusion: The widespread organ distribution of VSBV-1 point to the capacity of shedding via various routes and resembles closely the spread of mammalian 1 bornavirus in its reservoir, the bicolored white-toothed shrew (*Crocidura leucodon*). The dissemination of VSBV-1 over the entire body might be involved in its maintenance within the squirrel population, but imply also the risk for inter-species transmission. Thus, further investigations on potential transmission routes are urgently needed to address the potential animal and human health risk of this novel zoonotic virus.

NOTES

8

CHARACTERIZATION AND AETIOLOGICAL INVESTIGATION OF A NOVEL DISEASE OF RED SQUIRRELS WITH LEPROMATOUS DERMATITIS

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Introduction: Since 2006, a small number of cases of a novel dermatitis of red squirrels from Scotland has been reported as part of a scanning surveillance scheme. Since our first report, two additional well-defined locations with similar cases have been identified in England (*i.e.* Brownsea island and Isle of Wight).

Materials and Methods: Twelve dead red squirrel with dermatitis were collected, as well as 17 grossly normal Brownsea island squirrels. A gross post-mortem examination was conducted, and skin samples taken (muzzle, eyelids, pinna, forelimb, hindlimb). The latter were processed for histology (H&E and Ziehl Neelsen). Scottish samples were processed for PCR for *hsp*65 detection.

Results: Grossly, there was moderate to severe cutaneous swelling and alopecia involving the muzzle and pinna in all cases, with variable involvement of eyelids, tarsal and/or carpal, and urogenital areas. Histologically, these squirrels had focally extensive diffuse dermal infiltration by foamy macrophages containing large numbers of intracytoplasmic acid fast bacteria (AFBs). In addition, there was frequent neuritis (perineural and intraneural), with occasional intraneural AFBs. Unexpectedly, 6 grossly normal squirrels from an affected area also had mild chronic perineuritis, with intraneural AFBs in one squirrel. Sequencing of *hsp*65 PCR products from Scottish squirrels revealed a 99% homology with *Mycobacterium lepromatosis*.

Conclusions: Herein we describe a novel mycobacterial dermatitis of red squirrels in several UK locations. In Scottish cases this is associated with a reported human leprosy agent. Aetiological studies of all the available samples is ongoing.

NOTES

9

EUROPEAN RED FOXES (*VULPES VULPES*) AS A POSSIBLE RESERVOIR FOR CANINE ADENOVIRUS TYPE 1 (CADV-1)

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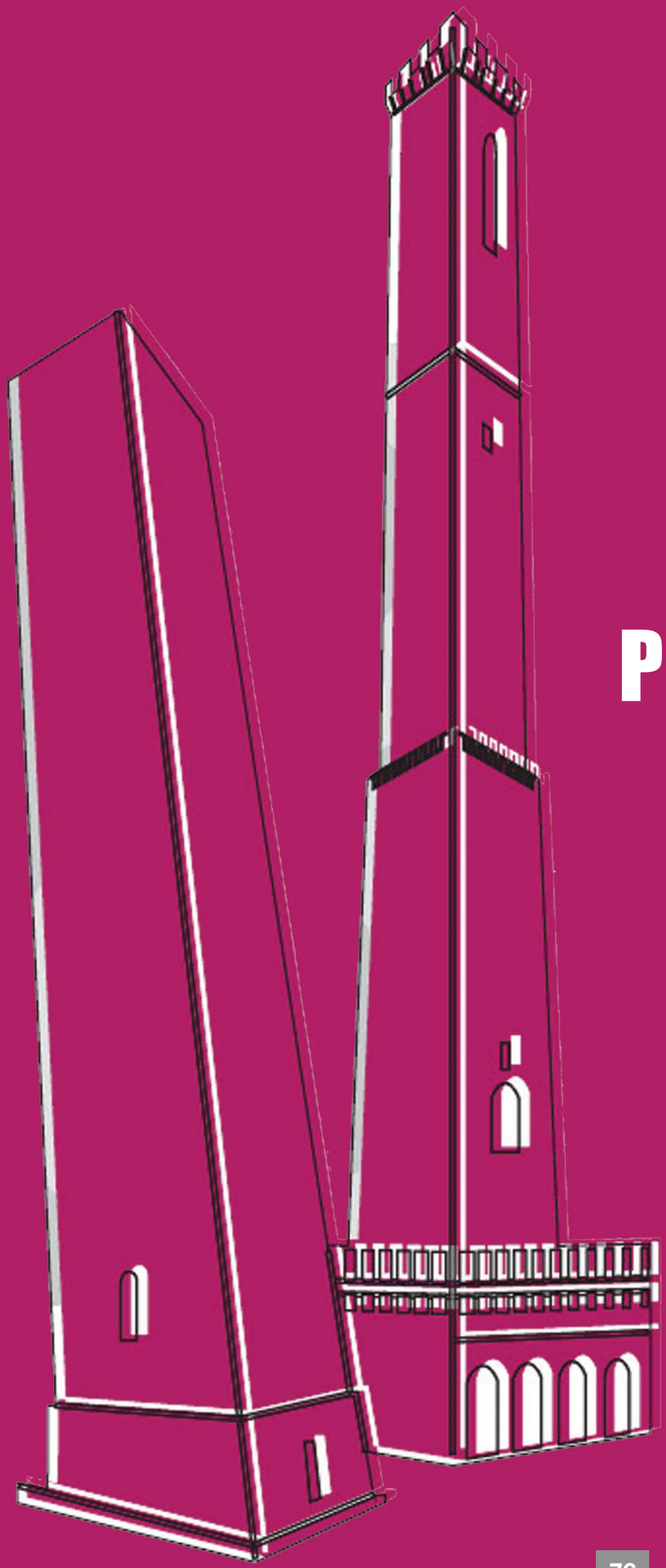
Introduction: In European red foxes (*Vulpes vulpes*), CAdV-1 infection is occasionally reported as a cause of illness (epizootic fox encephalitis and infectious canine hepatitis) and evidence of exposure to CAdV-1 has been confirmed in different European countries.

Materials and Methods: Fifty-seven red foxes of both genders (38 males and 19 females) and different ages (11 juveniles and 46 adults), shot during the regular hunting season in central Italy (36 animals) and the North-West of England (21 animals) underwent full post mortem examination. A complete set of organs was sampled for histopathology, hepatic and renal samples were tested for CAdV by PCR and immunohistochemistry (IHC).

Results: All foxes were in fair to good body condition with 12/57 (21%) presenting different degrees of sarcoptic mange. PCR performed on renal samples showed CAdV-1 specific amplicon, encompassing E3 gene and flanking regions, in 18/57 foxes (31.5%) with similar prevalence in both Italian and UK subjects. Hepatic samples were always PCR-negative. Histopathology did not reveal any lesions suggestive of CAdV-1 and IHC was negative in hepatic and renal samples. Four positive amplicons (two Italian and two English) were sequenced with high identity rate of 99.9% and phylogenetic analysis confirmed CAdV-1 homology for all the sequences analysed.

Conclusion: The presence of CAdV-1 infection in foxes could represent a problem for both wild animals and domestic dogs. Our data confirms the central role of red foxes in Europe play as a possible reservoir rather than as an incidental host in maintaining this virus in the territory.

NOTES



ORAL PRESENTATIONS

Session C - Neuropathology

10

INVESTIGATIONS INTO THE ROLE OF EXTRACELLULAR MATRIX IN CANINE DISTEMPER DEMYELINATING LEUKOENCEPHALITIS

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Introduction: In demyelinating diseases, like multiple sclerosis in man, changes in the quality and quantity of molecules of the extracellular matrix (ECM) may lead to loss of myelin and axons and could contribute to insufficient regeneration. According to this, there is only limited Schwann cell-mediated remyelination in canine distemper virus- induced demyelinating leukoencephalitis (CDV-DL). However, the role of the ECM is still uncertain.

Materials and Methods: CDV-DL lesions in formalin-fixed and paraffin-embedded tissue of animals naturally infected with the virus were classified according to morphological criteria, antigen detection and myelin loss. Additionally, comparative analysis of genes encoding for ECM molecules as well as enzymes for degradation and modification of ECM were done. Furthermore, immunohistochemical investigations of ECM molecules were performed.

Results: Microarray analyses revealed only few differentially expressed ECM-associated genes in CDV lesions compared to controls. Immunohistochemically, an increased amount of aggrecan was detected in early and late white matter lesions. In addition, the positive signals for collagens I and IV as well as fibronectin were increased in late lesions. Conversely, the expression of phosphacan was decreased in early and especially in late lesions compared to controls.

Conclusions: The observed findings indicate that changes in the quality and content of ECM molecules represent important features in advanced lesions of CDV-DL. Considering the insufficiency of morphological regeneration in chronic lesions, the demonstrated accumulation of ECM molecules might play a crucial role, potentially explaining the small regenerative potential in late stages of this disease.

NOTES

11

JAPANESE ENCEPHALITIS VIRUS TROPISM AND VECTOR FREE TRANSMISSION IN EXPERIMENTALLY INFECTED PIGS

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Introduction: Japanese encephalitis virus (JEV), a main cause of human encephalitis, has a complex ecology, composed of a cycle involving waterbirds and mosquitoes as well as pigs as amplifying hosts. The pathogenesis of JEV in pigs however remains obscure, especially with regard to outbreaks in pigs without evidence of virus carrying mosquitos. Here, we investigated the localization and kinetics of virus replication and potential direct transmission between in pigs.

Materials and Methods: Several successive animal experiments were performed under BSL3 conditions. Altogether 36 healthy specific-pathogen-free, 7-week-old Swiss Large White pigs were experimentally infected by i.V. and i.D. injection (18) or oronasally (18) using JEV strains Nakayama and Laos. 8 uninfected animals were housed in contact to infected animals. Daily, clinical signs were recorded and oronasal swabs and blood samples were taken. 7 – 25 days p.i. animals were euthanized, necropsied, viral RNA was quantified in organ samples and histopathological evaluations were performed.

Results: Clinical symptoms, virus tropism and CNS lesions were similar regardless of the route of inoculation. Pigs shed virus in oronasal secretions and were highly susceptible to oronasal infection. A particular tropism of JEV in pigs to the CNS and the tonsils was observed. In the latter JEV persisted for at least 25 days despite the presence of high levels of neutralizing antibodies. Importantly, we also demonstrated that JEV can be transmitted between pigs in the absence of arthropod vectors.

Conclusions: Our findings suggest that pigs could serve as reservoirs for JEV in temperate regions with short mosquito seasons.

NOTES

12

DETECTION OF A NEW PESTIVIRUS IN THE CENTRAL NERVOUS SYSTEM OF PIGLETS WITH CONGENITAL TREMORS

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Introduction: Congenital tremor in piglets represents a syndrome which is clinically characterized by rhythmic shivering increasing in severity during excitement and decreasing while animals are sleeping. Recently an atypical porcine pestivirus was detected in the serum of healthy pigs in the US. The aim of the present study was to investigate the occurrence and distribution of atypical porcine pestivirus (APPV) in piglets affected by congenital tremor.

Materials and Methods: Six two-day old piglets exhibiting congenital tremor as well as two age-matched, clinically unremarkable piglets from the same herd were investigated macroscopically and histologically. In addition, quantitative reverse transcription PCR (qRT-PCR) and *in-situ* hybridization (ISH) were used for the detection of APPV genome.

Results: Histologically, lesions were restricted to a mild myelin loss in the spinal cord in four out of six shivering piglets using Luxol fast blue staining. In all clinically affected piglets a fragment of the NS3 coding region of APPV was detected in cerebellum, spinal cord, ganglia, glands of the *arcus palatoglossus*, lymph nodes, and gastrointestinal tract using qRT-PCR and ISH while no APPV genome was detected in non-diseased piglets.

Conclusions: The presents study shows the association of APPV with congenital tremor in neonatal piglets. However, since histological lesions in the spinal cord were mild and not present in all APPV-positive animals the pathogenic mechanism of this newly identified pestivirus maybe unrelated to the observed myelin loss and should be investigated in detail in future studies.

NOTES

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A PESTIVIRUS DIVERGENT FROM APPV ASSOCIATED WITH MYOCLONIA CONGENITA IN PIGLETS

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Introduction: Piglets with myoclonia congenita were born in multiple litters in a pig breeding farm in Austria. Prevalence of affected animals was up to 100% in some litters.

Materials and Methods: Necropsy was done on six clinically affected piglets out of two litters and two clinically healthy littermates. Brain, spinal cord and organ samples of all animals and a healthy control were taken for histology and stained with H&E and LFB-H&E. To detect pestivirus a degenerated RT-PCR protocol targeting NS5B gene and for IHC a cross reacting monoclonal antibody against the E2 was used.

Results: Clinically affected animals showed an atypical horizontal movement of the head and generalized tremor. In all animals, including healthy littermates, severe bilateral symmetrical vacuolization of the cerebellar white matter was evident. Scattered vacuoles were detectable in nerve tracts and nuclei of formatio reticularis and trigeminal nerve. Due to severe hypo-myelination the cross sectional area of the spinal cord was reduced in all animals compared to a healthy control. A novel pestivirus was identified by PCR. From sera and various tissues virus was isolated and propagated on SK6 cells without obvious cpe. Pestivirus antigen was detected by IHC in kidneys, tonsils, retina, spinal ganglia, and in CNS areas showing alterations in HE-staining.

Conclusions: Animals infected with this pestivirus showed slightly different symptoms and consistent lesions in brain and spinal cord. The novel pestivirus is clearly distinct from the recently identified APPV/CTV as well as from all other pestivirus species.

NOTES

14

INCREASED EXPRESSIONS OF ADAMTS-13, 8-OHdG AND GFAP-NF CORRELATE WITH NEUROPATHOLOGY IN FELINE INFECTIOUS PERITONITIS VIRUS-INFECTED CATS

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Introduction: It is seen that the coronaviruses cause widespread disease in humans and animals. Feline infectious peritonitis (FIP) is a lethal systemic granulomatous disease in the cat population worldwide caused by FIP virus (FIPV). There is no study focusing on investigations of A Disintegrin And Metalloprotease with Thrombospondin type I repeats-13 (ADAMTS-13) expression and oxidative stress-related neuropathology in FIPV infection. The aim of this study was to identify the cytotoxic effects of oxidative stress and ADAMTS-13, glial fibrillary acidic protein (GFAP)-neurofilament (NF) expressions and to identify whether they have any correlation with FIP neuropathology.

Materials and Methods: Expression levels of ADAMTS-13, 8-hydroxy-2'-deoxyguanosine (8-OHdG), GFAP and NF in FIPV-infected brain tissues from cats (n=13) were evaluated immunohistochemically.

Results: Levels of ADAMTS-13 ($P<0.001$), 8-OHdG ($P<0.001$), GFAP ($P<0.001$) and NF ($P<0.001$) expressions remarkably increased in FIPV-infected cats versus healthy control.

Conclusions: The most prominent finding from our study was that ADAMTS-13 and GFAP increased significantly and this may play an important role in the protection and regulation of the blood-brain barrier integrity and central nervous system (CNS) microenvironment in this disease. Furthermore, expression of NF and ADAMTS-13 might gives an idea of the progress and critical for diagnostic significance of this disease. These results also suggest that FIPV-mediated oxidative stress might play a pivotal role and a different type of role in immunopathogenesis in the process of neurological FIP. To the best of the authors' knowledge, this is the first report on NF and ADAMTS-13 expression in the CNS of FIPV-infected cats.

NOTES

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INCREASE OF COLONIC MOTILITY IN CATS AFFECTED BY CHRONIC IDIOPATHIC CONSTIPATION AND MEGACOLON AFTER THERAPHY WITH THE PROBIOTIC SIVOY™

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Introduction: The pathogenesis of chronic constipation (CC) and idiopathic megacolon (IMC) has been poorly understood in humans and animals. CC and IMC occurs more often in the cat than the dog. Actually it is unknown whether there are abnormalities involving the extrinsic nerves, the enteric nerve plexuses, interstitial cells of Cajal (ICC) or the intestinal smooth muscle. In humans, probiotics have been increasingly investigated in the management of these colonic motility dysfunctions, particularly their effect on gut transit time, stool output, and constipation.

Materials and Methods: To investigate clinical and histological effects of a commercial multi-strain probiotic (SIVOY™), 10 privately owned chronically constipated cats of different breeds and ages, non-responsive to medical management, were selected on the basis of recurrence of clinical signs and absence of any antibiotic treatment for a month. Three cats suffered from IMC and full thickness biopsies were sampled for histology. In all animals enrolled in the study, the colon was found to be dilated and impacted with feces. CC (n=7) and IMC (n=3) cats received orally 200 billion lyophilized bacteria daily for 90 days.

Results: Constipated cats displayed a significant decrease in ICC, and cats with IMC had significantly more apoptotic enteric neurons than controls. After treatment with SIVOY™, significant decreases were observed for FCEAI clinical index ($p=0.006$), and histology scores ($p<0.001$). In contrast, a significant increase of CD117+ ICC was observed ($p<0.05$) after probiotic therapy.

Conclusions: Histological parameters suggest a potential anti-inflammatory effect of SIVOY™, associated with a reduction of mucosal infiltration, and restoration of the number of ICC.

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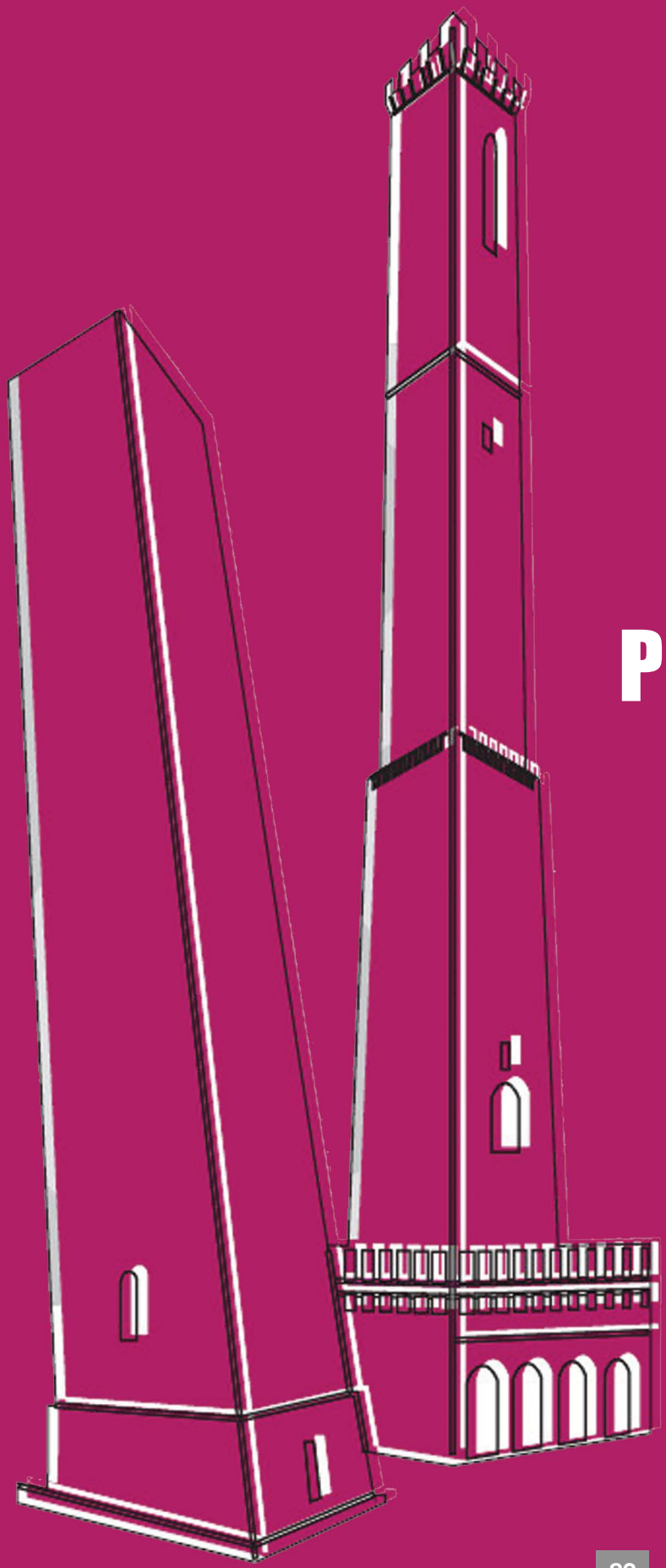

ORAL PRESENTATIONS

Session D - Oncology

16

TRANSCRIPTOME ANALYSIS OF CANINE CUTANEOUS MELANOMA AND MELANOCYTOMA REVEALS A MODULATION OF GENES INVOLVED IN COLLAGEN METABOLISM AND CELL SURVIVAL

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Introduction: The interactions between tumour cells and tumour microenvironment are considered critical in carcinogenesis, tumour invasion and metastasis. The identification of melanoma-specific deregulated genes could provide molecular markers for further insight into melanoma tumorigenesis.

Material and Methods: Total RNA extracted from formalin fixed, paraffin embedded (FFPE) specimens from canine cutaneous melanomas (n=4) and melanocytomas (n=4) was analysed by means of RNA-Seq with directional libraries for transcriptional analysis. After cleaning procedures, reads were aligned to the reference genome (CanFam3.1). Differential gene expression analysis through a count-based approach was applied comparing melanocytomas versus melanoma samples. Gene networks and significant enriched pathways were explored with different packages (StringDB, pantherdb and CluedGO/Cluedpedia) to reveal functional relations between differentially expressed genes.

Results: The results of our experiment demonstrate that there is a differential expression of 60 genes in melanomas compared to melanocytomas. These genes cluster in the extracellular matrix-receptor interaction, protein digestion and absorption, focal adhesion and PI3K-Akt signalling pathways. In particular, genes encoding for several collagen proteins were differentially expressed.

Conclusions: We hypothesize that the developing melanoma actively promotes collagen metabolism and extracellular matrix remodelling as well as enhances cell proliferation and survival mechanisms contributing to disease progression and metastasis. In this study, we also detect genes not yet identified in human melanoma expression studies and uncover new candidate drug targets for further testing in canine cutaneous melanoma.

NOTES

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ATYPICAL PHENOTYPES IN CANINE LYMPHOMAS: THE MORE YOU SEARCH THE MORE YOU FIND

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Introduction: Lymphoma is one of the most common neoplasms encountered in canine species and its phenotype can provide prognostic and predictive information. At present, phenotype is assessed mainly with the use of immunohistochemistry, and CD3 and CD79a are the most used markers for this purpose. Traditionally CD3+/CD79- or CD3-/CD79+ warrant the diagnosis of T or a B lymphoma respectively, while CD3-/CD79- immunophenotype, together with the negativity for other round cell markers is suggestive of a non-B non-T (null) lymphoma.

Materials and Methods: A retrospective analysis of CD3- CD79a- (Null) lymphoma cases was conducted and cases submitted to a comprehensive immunohistochemical panel including CD20, PAX5, MUM1 for B lineage. CD18, Iba1, CD117 and mast cell tryptase were used to exclude different round cell origins. Furthermore a selection of T lymphoid neoplasms previously diagnosed by CD3 labelling but exhibiting aberrant patterns of expression of B markers were selected.

Results: Among five cases of CD3-/CD79a- lymphomas retrieved, three cases expressed alternative B cell markers, making possible a re-classification into B cell lymphomas. In addition, two T lymphomas were observed expressing MUM1, and two epitheliotropic T cell lymphomas were CD3+/CD20+/CD79a+, showing a double B and T phenotype which was confirmed in one case with clonality test.

Conclusions: A subpopulation of CD3- CD79a- lymphomas are indeed B cell lymphomas which express alternative B markers to CD79a, suggesting an early or terminal B cell maturation. Rare T cell lymphomas exhibit MUM1 or CD79a/CD20 expression and in some cases this is indicative of a genuine double clonality.

NOTES

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HISTOLOGIC CHARACTERIZATION OF 100 URINARY BLADDER AND URETHRAL TRANSITIONAL CELL CARCINOMAS IN DOGS FROM THE UNITED KINGDOM

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Introduction: Transitional cell carcinoma (TCC) is the most common form of urinary tract cancer in the dog and comprises 1.5 to 2% of all canine cancers. The aim of this study was to histologically and retrospectively characterize canine urinary TCC and to assess the suitability of the current human consensus classification of the 2004 World Health Organization (WHO)/1998 International Society of Urological Pathology (ISUP).

Materials and Methods: One hundred canine urinary bladder (n=77) and/or urethral (n=27) TCC were available. The samples were taken from 26 different dog breeds, and included 68 females, 31 males and one dog with unknown sex. All tumours were histologically characterized and categorized in accordance with the WHO/ISUP human consensus classification system.

Results: In order of their frequency, the tumours were classified as: Infiltrating urothelial carcinoma (IUC) (n=79), IUC with glandular differentiation (n=8), IUC with squamous differentiation (n=4), non-invasive papillary urothelial carcinoma low grade (n=3), non-invasive papillary urothelial carcinoma high grade (n=1), non-invasive urothelial carcinoma in situ (n=1), inverted urothelial papilloma (n=1). In addition, one case was suspected of microcystic IUC, two cases of IUC showed both glandular and squamous differentiation, and one case consisted of an IUC, urothelial papilloma and inverted papilloma. In 20% of the cases, certain histologic features (e.g., mucoid stroma and comedonecrosis) were prominent.

Conclusions: Results suggest that the application of the WHO/ISUP human classification system to canine urothelial neoplasms is suitable. More canine TCC will be assessed (over 250 are currently available) in order to confirm and expand current preliminary results.

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CLINICOPATHOLOGICAL CRITERIA FOR CUTANEOUS MAST CELL TUMOUR GRADING IN CATS

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Introduction: Mast cell tumours (MCTs) account for up to 21% of cutaneous neoplasms in cats. There is currently no grading system for feline cutaneous MCTs and prognosis is difficult to estimate. Although the typical form is usually a benign tumour that can be cured by complete surgical excision, a small but important proportion of MCTs are biologically aggressive and will spread to local lymph nodes or visceral sites within months of excision.

Materials and Methods: A series of macroscopic and histological features was evaluated in a population of feline cutaneous MCTs treated with surgical excision plus or minus adjuvant chemotherapy and available long-term follow-up information. The criteria that best correlated with prognosis were used to develop a grading scheme to predict outcome.

Results: Forty-eight feline cutaneous MCTs were included in the study. Thirteen of them died of tumour-related disease (median survival time, 476 days), while the remaining 35 were still alive after 1000 days from surgical excision. Tumours were classified as high grade in the presence of at least 4 of the following 6 criteria: tumour diameter >1 cm, high nuclear-cytoplasmic ratio, irregular nuclear shape (nuclear pleomorphism), 2-fold variation of nuclear diameters (anisokaryosis), prominent nucleoli or >5 mitotic figures/10 HPFs. According to this scheme, 15 cases (31.2%) were classified as high grade MCTs.

Conclusions: High grade feline cutaneous MCTs were significantly associated with metastatic spread and reduced survival time. The median survival time was 589 days for high-grade MCTs and 1498 days for low-grade MCTs ($P < 0.0001$).

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TARGETING G_0-LIKE RESIDUAL CELLS IN A MOUSE MODEL FOR BRCA1-DEFICIENT MAMMARY TUMORS

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Introduction: Residual disease is a major clinical problem in cancer therapy. To study the underlying mechanisms we are using a genetically engineered mouse model for BRCA1-associated breast cancer (*K14cre;Brca1$^{F/F}$;p53$^{F/F}$*). Like in patients, some residual cancer cells escape the anticancer therapy, despite repeated sensitivity to DNA damage-inducing agents. In our model we identified a subpopulation of G_0-like cells, which is more resistant to anticancer therapy and appears to cause tumor relapse. By blocking inhibitory T-cell signaling we aimed to increase the activity of T-cells towards the G_0-like cells and thereby eradicate tumors.

Materials and Methods: Mice bearing BRCA1-deficient mouse mammary tumors were treated with the PARP inhibitor olaparib or cisplatin alone or in combination with CTLA-4 and PD-1-targeting antibodies. The time until the tumors relapsed was monitored (n=15 per group) and tumor infiltrating lymphocytes in residual tumors (n=5 per group) were quantified using IHC.

Results: Combination of CTLA-4 and PD-1-targeting antibodies with olaparib led to an increase of CD3-positive T-effector cells (Teffs) in residual tumors. Surprisingly, this increase did not result in a therapeutic benefit. Intriguingly, the number of intratumoral FoxP3-positive T-regulatory cells (Tregs) increased, while we expected a depletion of Tregs.

Conclusions: We were not able to eradicate the residual tumor cells using immunotherapy. A possible explanation may be an adverse effect of increasing Tregs. A better understanding of how Tregs accumulate upon checkpoint inhibition may be helpful to understand why immunotherapy fails. Furthermore, characterization of the G_0-like residual cells may elucidate novel therapeutic targets to eradicate BRCA1-deficient mammary tumors.

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CORRELATION BETWEEN CYTOLOGY, HISTOPATHOLOGY AND IMMUNOHISTOCHEMISTRY IN THE DIAGNOSIS OF CANINE CUTANEOUS AND SUBCUTANEOUS MASSES

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Introduction: Cytological diagnosis is a rapid adjunct to histopathological examination. However, the results of cytological examination need to be confirmed with histopathology and in some cases with immunohistochemistry.

Materials and Methods: A total of 71 canine cutaneous and subcutaneous masses were diagnosed cytologically and histopathologically. Additional immunohistochemical stainings were performed in 45 cases.

Results: Cytological diagnoses included 56 tumors (21 mesenchymal, 16 round cell, 15 epithelial, 4 melanocytic), 13 inflammatory reactions and 2 cysts. Of the 21 cytologically diagnosed mesenchymal tumors, three were later confirmed non-tumoral (fibroepithelial polyp, hematoma, granulation tissue). One mast cell tumor was confirmed to be fibrous hyperplasia; diagnoses were correct in other round cell tumors. Cytological diagnoses were correct for all melanocytic tumors and cystic lesions. Thirteen epithelial tumors were correctly diagnosed cytologically, whereas two cases were confirmed to be non-tumoral (fibroepithelial polyp and granulation tissue) after histopathological examination. Five cases which were cytologically diagnosed as inflammatory reaction were diagnosed as tumors (lymphoma, papilloma, sebaceous adenoma and two cases of squamous cell carcinoma) after histopathological examination. Histopathological diagnoses of all round cell tumors and epithelial tumors were confirmed with immunohistochemistry, while diagnoses changed in six mesenchymal tumors after immunohistochemical examination. Total accuracy of cytology in diagnosis of tumoral and non-tumoral character was 84.5% and in determination of benign or malignant behavior was 83%.

Conclusions: High success rates obtained with cytology proves that the method can be a reliable diagnostic tool. The main diagnostic challenge remains with mesenchymal tumors and tumors with concurrent inflammatory reactions.

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ORAL PRESENTATIONS

Session E - Mammary tumours

22

BCL-2 EXPRESSION IN FELINE INVASIVE MAMMARY CARCINOMAS IS ASSOCIATED WITH BETTER SURVIVAL

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Introduction: We have previously demonstrated that BCL-2 positivity in canine mammary carcinomas is associated with better prognosis (better overall survival and disease-free interval), as in human breast cancer. The aim of this study was to determine the frequency and prognostic value of BCL-2 expression in feline invasive mammary carcinomas (FMCs).

Materials and Methods: Retrospective study of 180 FMCs, diagnosed in female cats treated by surgery only, whose outcome is known 2 years post-mastectomy. BCL-2 (clone 7/Bcl-2), ER (clone C311), PR (clone 10A9), Ki-67 (clone MIB1) and HER2 (clone 4B5) expression were determined by automated immunohistochemistry. BCL-2 expression was quantitated as an index.

Results: The cohort comprises 32% (57/180) luminal FMCs defined by ER and/or PR positivity, and 68% (123/180) triple negative FMCs (negative for ER, PR and HER2). BCL-2 expression was considered as positive when at least 65% of tumor cells were immunohistochemically stained. 18% (32/180) of the 180 FMC were BCL-2-positive independently of their luminal versus triple negative phenotype. By multivariate survival analysis, BCL-2 positivity (HR=0.60) in FMCs was associated with longer overall survival with the following covariates: pathologic tumor size (HR=1.67 for T larger than 20mm), nodal stage (HR=0.44 for N0 cases), distant metastasis (HR=2.95 for M1) and peritumoral inflammation (HR=0.71 when absent or low) ($P<0.0001$; Cox proportional-hazards regression).

Conclusions: As in humans and dogs, BCL-2 positivity is associated with better survival in cats with FMCs, although the thresholds of positivity are noticeably different in cats (65%) than in humans and dogs (10%).

NOTES

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MAST CELLS AS TARGETS ON MAMMARY CARCINOGENESIS

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Introduction: The role of mast cells on mammary carcinogenesis is not completely understood. The present work aimed to better understand the role of mast cells on mammary cancers which were chemically-induced in female rats.

Materials and Methods: Experiments were approved by Portuguese (no.008961) and University (CE_12-2013) Ethics Committees. Thirty female Sprague-Dawley rats were divided into three experimental groups. Mammary tumors were induced in all animals by the intraperitoneal injection of *N*-methyl-*N*-nitrosourea (MNU) at seven weeks of age. Animals from group I received water. Group II was treated with ketotifen (1mg/kg, drinking water) immediately after the MNU administration for 18 weeks; animals from group III received the ketotifen after the development of the first mammary tumor. Mammary tumors were evaluated by histopathology and immunohistochemistry (Ki-67, caspase-3 and -9) and proliferation and apoptotic indexes were determined.

Results: One animal from group II died during the experiment. Six animals from group I (60%), eight animals from group II (89%) and seven animals from group III (70%) developed mammary tumors. Fifty-eight mammary tumors were counted (21 in group I, 19 in group II and 18 in group III). Histologically, each mammary tumor exhibited more than one mammary lesion. Thirty-five lesions were identified in group I, 44 lesions in group II and 48 lesions in group III. The lowest proliferation and apoptotic indexes were observed in group II.

Conclusions: The mainly positive effect of the mast cells inhibition seems to be the reduction of tumors proliferation when the mast cells' degranulation was inhibited before tumors development.

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HER2 GENE AMPLIFICATION STATUS IN FELINE MAMMARY CARCINOMA: FLUORESCENCE *IN SITU* HYBRIDIZATION ANALYSIS

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[1]Both authors contributed equally to the work.

Introduction: ErbB2 is a tyrosine kinase receptor overexpressed in a subset of breast cancer due to the *HER2* gene amplification. ErbB2 is expressed in feline mammary carcinomas (FMC), but little is known about the cytogenetic alterations. The aims of this study were to evaluate the *HER2* gene amplification status and its correlation with the erbB2 protein expression in FMC.

Materials and Methods: FMC were retrospectively selected and immunohistochemically evaluated for erbB2 expression. ErbB2 positive (3+) and equivocal (2+) cases were selected for Fluorescence *in situ* hybridization (FISH), and a subset of negative cases (0/1+) were used as negative control. Dual-core tissue microarrays were prepared for FISH. IHC and FISH were evaluated according to the ASCO/CAP guidelines.

Results: 110 FMC from 88 queens were selected. ErbB2 expression was positive (3+) in 8 cases, equivocal (2+) in 49 cases, and negative (0/1+) in 53 cases. *HER2* status was indeterminate in 6 FMC, amplified in 3 (2 erbB2 positive and 1 equivocal), equivocal in 4 (2 erbB2 positive and 2 equivocal), non-amplified in 55 (4 erbB2 positive, 41 equivocal and 10 negative). *HER2* amplification and erbB2 expression were significantly correlated ($R=0.28$; $p<0.05$).

Conclusions: *HER2* is amplified only in a subset of FMC despite the erbB2 positive or equivocal expression. FISH is a specific method to identify the *HER2* amplification in positive and equivocal cases assessed by IHC, and it may provide useful data for therapeutic purposes.

NOTES

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THE WNT/BETA-CATENIN PATHWAY IN CANINE MAMMARY CARCINOMA AS A PONTENTIAL TARGET OF ANTI-CANCER DRUGS: AN IN VITRO STUDY

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Introduction: Mammary tumors are the most common tumors of intact female dogs. In aggressive and advanced mammary cancers, where metastases have occurred, surgery is not curative alone. Hence, it seems to be extremely important to identify new therapies for canine mammary carcinomas (CMCs). Several tumorigenic pathways, such as the Wnt/beta-Catenin pathway, have been studied to identify new potential therapeutic targets. Specifically, the natural alkaloid barberine (BBR) and its derivatives have been recently described as targeting the Wnt/beta-Catenin signaling with a promising anticancer activity.

Materials and Methods: We tested the effect of BBR and of nine derivatives on the CMC CF33 cell line. Twenty-four hours after seeding, cells were treated for 24h with the compounds, at different concentrations. Cell viability was analyzed by TOX8 assay. Flow cytometry analysis was applied in order to assess if the arrest of proliferation and cell death was due to apoptosis or necrosis. Zebrafish was used as a model for drug toxicity test. Moreover, zebrafish reporters were used to dissect the molecular mechanism affected by these compounds.

Results: Three out of ten compounds were identified to be able to significantly affect the proliferation of CF33 cell line, mainly by the activation of apoptosis. Remarkably, the molecular mechanism that appeared to be involved in the arrest of CF33 cell proliferation was confirmed to be the Wnt/beta-Catenin pathway.

Conclusions: The Wnt/beta-Catenin pathway appeared as a relevant pathway in mammary cancer cell proliferation in the dog, and apparently it could be targeted by three BBR derivatives, thus suggesting their possible use as anti-cancer drug.

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PROTEOMIC DISCOVERY AND IMMUNOHISTOCHEMICAL VALIDATION OF GLUCOSE METABOLISM-RELATED ENZYMES AS BIOMARKERS IN CANINE MAMMARY TUMOURS

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Introduction: The identification of cancer biomarkers and their translation to clinical relevant assays represent a major area of investigation in canine mammary tumour (CMT). However, proteomic approaches have been rarely employed to identify biomarkers in CMT. The aim of this study was to discover and to validate differentially expressed proteins as candidate biomarkers in normal mammary gland and in CMT.

Materials and Methods: Three formalin-fixed tubulopapillary mammary carcinomas and 3 normal mammary glands were subjected to protein extraction, polyacrylamide gel electrophoresis, and trypsin in-gel digestion. Peptide mixtures were separated by liquid chromatography, analyzed by tandem mass spectrometry and pathway-categorized through Ingenuity Pathway Analysis. Immunohistochemical (IHC) expression of Transketolase (TKT) and Transketolase Like 1 (TKTL1) proteins were analyzed in normal ($n = 6$) and in hyperplastic glands ($n = 3$), in benign ($n = 11$) and in malignant mammary tumours ($n = 17$).

Results: A list of 40 differentially expressed proteins, mainly involved in cell proliferation and in cancer development, was discovered by proteomic analysis. Among them, two glucose metabolism-related proteins, TKT and TKTL1, were selected and validated by IHC. A significantly increased expression of TKT was observed in hyperplastic and neoplastic lesions compared with normal mammary glands by IHC, suggesting a pivotal role of this protein in mammary carcinogenesis ($P < 0.05$). TKTL1 was higher in hyperplastic lesions, simple adenomas and carcinomas than in normal mammary glands ($P < 0.05$).

Conclusions: The present study revealed that metabolic changes are critical in tumor development and TKT and TKTL1 are valid glucose-related biomarkers in CMT.

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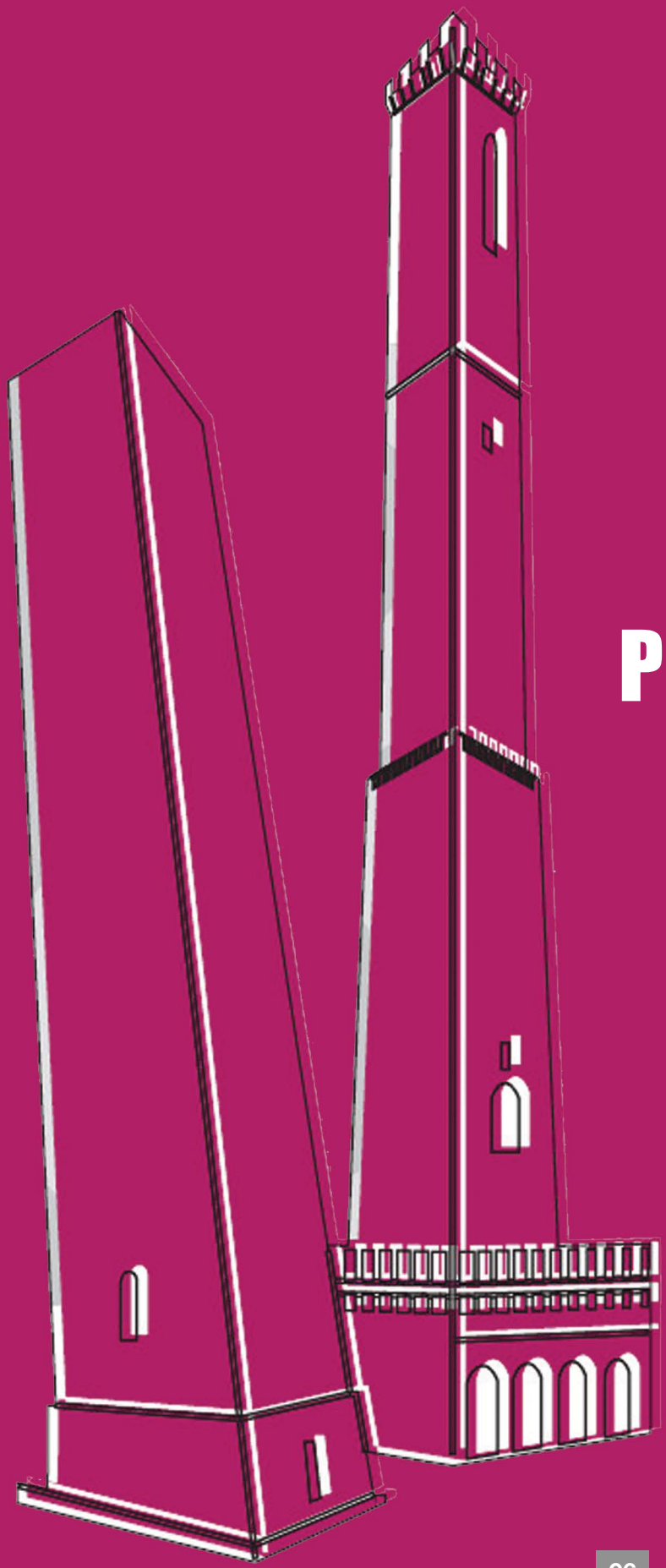

ORAL PRESENTATIONS

Session F - Miscellaneous

27

OXALATE NEPHROSIS IN CAPTIVE CHEETAHS (*ACINONYX JUBATUS*): PATHOLOGY FINDINGS

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Introduction: Oxalate nephrosis accounts for approximately 10% of captive cheetah deaths. This study characterized of the epidemiology and pathology of the condition to facilitate prevention, diagnosis and treatment.

Materials and Methods: Renal and gastrointestinal lesions, age, gender, institution and country were recorded in captive cheetahs with (n=100) and without (n=165) oxalates and analysed using mixed effects logistic and negative binomial regression models for the presence and number of oxalate crystals, respectively.

Results: Raman spectroscopy confirmed that the crystals were oxalates. Both the presence and number of oxalates were positively associated with indicators of tubular damage. Amyloidosis, renal interstitial nephritis and colitis were positively associated with the presence of oxalate crystals, whereas glomerular loop thickening and gastritis were associated with reduced odds of having oxalates. In contrast, glomerulosclerosis, amyloidosis and interstitial nephritis scores were each independently inversely associated with the number of crystals. Colitis was not associated with crystal number. Crystal number was inversely associated with age, and was lower in African than American and European animals. Most of the cheetahs with the highest number of crystals were from North America.

Conclusions: Oxalate nephrosis unrelated to ethylene glycol toxicity should be considered as a diagnosis particularly in young cheetahs that are less likely to be suffering from amyloidosis or glomerulosclerosis. Oxalate nephrosis in cheetahs is of uncertain aetiology but is not directly related to other causes of renal disease, nor to liver disease, gastritis or enteritis. A multifactorial pathogenesis including a primary genetic predisposition, diet and, potentially, colitis is suspected.

NOTES

28

GENERATION OF AEROSOLS DURING BONE SAWING PROCEDURES IN VETERINARY AUTOPSIES AND EFFECTIVE PREVENTIVE MEASURES FOR THE PROTECTION OF AUTOPSY WORKERS

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Introduction: Aerosols generated during bone sawing processes are a health risk for veterinary pathologists. The aim of this study was to demonstrate the aerosol production and its distribution inside a necropsy facility during routine bone sawing procedures and to demonstrate the effectiveness of constructing a separately ventilated sawing room in reducing aerosol exposure.

Materials and Methods: The experiments were carried out with 3 different types of saws: An industrial stationary bone band saw MKB-653 (MADO GmbH), an oscillating autopsy saw HB-740 (KUGEL medical GmbH) and a standard butcher bone hand saw (F. Dick GmbH). Aerosolized particles of >0.3, >0.5, >0.7, >1, >5, and >10 µm were detected with the laser diode particle counter CI-7300 (CLIMET Instruments). All tests were performed under conditions simulating regular practical work.

Results: The band saw produced vast amounts of aerosols which spread rapidly across the entire autopsy hall. After constructing a separately ventilated sawing room, no aerosols were detectable outside this room. Inside this room, however, aerosol were still distributed throughout the entire room despite an improved ventilation system. Respirators limited the exposure of operators inside the sawing room. The oscillating saw and the hand saw generated smaller amounts of aerosol which could only be detected at the position of the operator.

Conclusions: Bone sawing procedures should be considered with care due to the potential aerosolization of zoonotic pathogens. Aerosol exposure of personnel in autopsy facilities can be prevented by appropriate design of the facility in combination with accurate personal protective devices.

NOTES

29

DEVELOPMENT OF DIFFERENT ANIMAL MODELS FOR MIDDLE EAST RESPIRATORY SYNDROME-CORONAVIRUS (MERS-CoV) INFECTION

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Introduction: Middle East respiratory syndrome coronavirus (MERS-CoV) emerged in 2012 as a causative agent of severe pneumonia and mortality in humans. Further research indicated that an animal reservoir for MERS-CoV did exist (dromedary camels). Since other animal species susceptible to this viral infection cannot be ruled out, the aim of this work was to test four different species for their susceptibility to MERS-CoV and potential as an animal model.

Materials and Methods: A total of 14 pigs, 14 sheep, 8 horses (ponies) and 8 llamas were intranasally inoculated with 10^7 $TCID_{50}$/animal. Four pigs and 4 sheep were euthanized on day 2 post-inoculation (PI) and 4 animals of each species were sacrificed on day 4 and on day 24 PI. A complete necropsy was performed on all animals, and histopathological and immunohistochemistry (IHC) assessment was done on a set of tissues with emphasis on the respiratory tract. Virus isolation and RT-PCR to detect MERS-CoV was performed on nasal swabs from several days PI (1, 2, 3, 5, 7, 10, 15 and 25).

Results: Only llamas showed clinical signs consisting of moderate nostril mucous secretion. Inflammation and necrosis was predominantly seen in the nasal respiratory epithelium of llamas and pigs. Only pigs and llamas displayed positive virus isolation and RT-PCR results in nasal swabs variably from days 1 to 10.

Conclusions: Pigs and llamas are susceptible to infection with MERS-CoV and might be used as potential models of subclinical infection with this virus.

NOTES

30

ENDOMETRIAL LESIONS UNDERLYING FELINE PYOMETRA

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Introduction: Pyometra is an important uterine disease in cats and usually secondary to other uterine lesions, often associated with cystic endometrial disease [cystic endometrial hyperplasia/ pyometra complex (CEH-Pyo)]. However, recent evidences points to the fact that pyometra also accompanies feline endometrial adenocarcinoma (FEA) lesions. Failure to properly analyse through histopathology any pyometra situation may permit the spread of a more aggressive undetected neoplasia. This work intented to draw the practitioners attention to the need for the careful histopathological examination of the excised feline genital tract particulalyr when areas of increased mural thickness are noted.

Material and methods: We evaluated the records of 271 feline ovariohysterectomy (OVH) specimens from laboratory archives within the last seven years.

Results: In 271 OVH specimens, 125 had lesions. CEH was the most frequent finding (44%), followed by pyometra (30.4%) and FEA (25.4%). Concomitant with pyometra we found FEA in 47.3% of cases. Only in 13.2% of cases pyometra was CEH observed simultaneously.

Conclusions: All OVH specimens should be carefully examined, as lesionsof FEA could be masked. FEA is usually described as a rare pathology in cats, but recent reports suggest that it may be more frequent than thought. Age is not an adequate factor for triage, since some FEA cases were described in young animals and pathologists and clinicians should be advised of this.

NOTES

31

17BETA-ESTRADIOL REGULATES OXYTOCIN PRE-PROPEPTIDE mRNA EXPRESSION IN CULTURED BOVINE SATELLITE CELL-DERIVED MYOTUBES

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Introduction: Estrogens induce Oxytocin (OT) expression in several tissues by classical and non-classical signaling. Aim of this study was to evaluate the role of Estrogen Receptor α (ERα) in OT mRNA expression in cultured bovine satellite cell (BSC)-derived myoblasts.

Materials and Methods: BSCs were isolated from deep digital flexor muscle of adult male cattle. At day 8, differentiation was induced and myotube development was demonstrated by specific IF staining. Myotubes at day 14 were treated with 17beta-estradiol (E) with and without ERα antagonist (ICI 182,780) for 24h and mRNA levels of OT pre-propeptide and Insuline-like Growth Factor 1 (IGF1) were measured by qPCR .

Results: OT mRNA level in myotubes was significantly up-regulated and this effect was not suppressed by ICI. IGF1 mRNA was mildly up-regulated by E treatment, and significantly up-regulated in E+ICI-treated myotubes.

Conclusions: E induces OT mRNA expression in BSC-derived myotubes, as previously described in bovine skeletal muscle *in vivo*. ICI exerts its antagonist activity by inhibition of the binding of the ERα-ligand complex to DNA, and it has been described as an activator of the orphan G-protein-coupled receptor 30 (GPR30). The IGF1 increase induced by E with ICI treatment is suggestive of GPR30 involvement. ICI does not influence OT over-expression induced by E, suggesting that E may exert its effect on OT transcription through *nuclear* orphan receptors. This is the first description of *in vitro* OT mRNA up-regulation induced by E in BSC-derived myotubes suggesting a role for OT in skeletal muscle differentiation.

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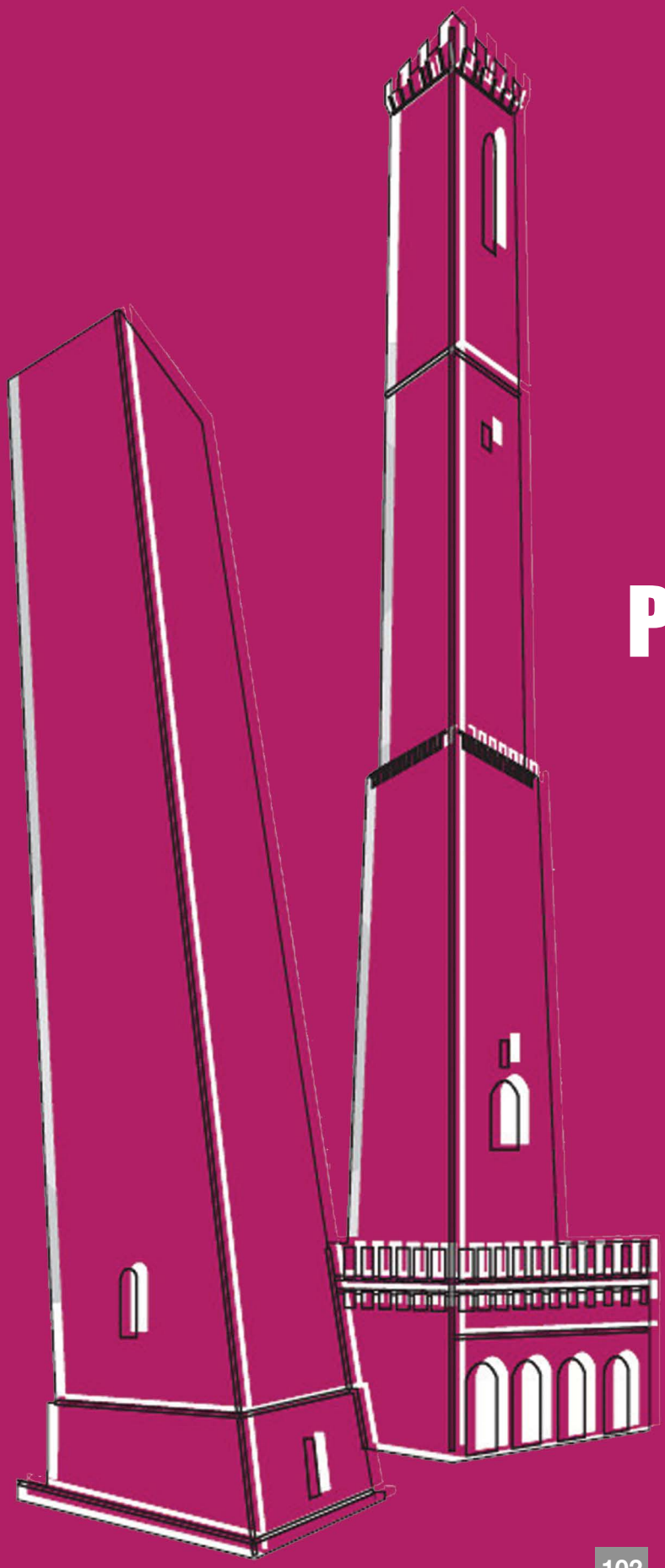

POSTERS FLASH

Session 1 - Oncology

1

TRUNCAL SKIN TUMOR DEVELOPMENT IN MICE WITH INDUCIBLE AND CONDITIONAL ONCOGENIC BRAF EXPRESSION AND PTEN DELETION UNDER THE TYROSINASE PROMOTER

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Introduction: the B6.Cg-*Braf*^{tm1Mmcm} *Pten*^{tm1Hwu} Tg(Tyr-cre/ERT2)13Bos/BosJ mouse represents an inducible Cre model where oncogenic *Braf* expression and *Pten* deletion are both controlled by the tyrosinase promoter. Despite the fact that the model is widely used to study *in vivo* the molecular basis of oncogenic BRAF-driven melanomagenesis, the exact cellular origin of tumor development and nature of its progression is far from being characterized.

Materials and Methods: this work combines sophisticated lineage tracing techniques with different reporters and detailed immunohistological analysis to elucidate origin of lesion development in the hairy skin parts (i.e. truncal skin) and study the pathobiology of the resulting tumors.

Results: lineage tracing experiment at early time points clearly identified the late anagen hair bulb as the main site of expansion of mutant melanocytes. At later stages, lesion evolution was characterized by the formation of poorly pigmented neoplasms with different degree of dermal-subcutaneous or fascial invasion but without any junctional epidermal involvement or metastasis. Microscopically tumors displayed neuroid configurations and growth patterns reminiscent of peripheral nerve sheath tumors. Expression of neural markers including GFAP, Nestin, MAP2 and SOX10 but lack of classical melanocyte markers, such as gp100, MITF and Trp1, was also demonstrated.

Conclusions: given the preferential targeting of hair bulb-associated melanocytes with lack of junctional epidermal involvement, divergent neural differentiation pathway of the resulting tumors (i.e. neurotization and/or Schwannian differentiation) and no metastasis, lesion development in the truncal skin of B6.Cg-*Braf*^{tm1Mmcm} *Pten*^{tm1Hwu} Tg(Tyr-cre/ERT2)13Bos/BosJ mice does not recapitulate the sequence of events that characterize the Clark's model human melanoma progression.

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2

BASAL CYTOKERATIN EXPRESSION IN FELINE INVASIVE MAMMARY CARCINOMAS

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Introduction: Most feline invasive mammary carcinomas (FMCs) express basal cytokeratins-5/6 (CK5/6) and -14 (CK14). In humans, basal cytokeratins may be used to define the "basal" phenotype of breast cancers, either luminal (positive to Estrogen Receptor ER and/or Progesterone Receptor PR) or triple negative (negative to ER, PR, and HER2 Human Epidermal Growth Factor Receptor-2). The purpose of this study was to assess the existence of "luminal-basal" and "triple negative-basal" FMCs, using CK5/6 and CK14 as basal cell markers.

Materials and Methods: Retrospective study of 350 FMCs with 2-year follow-up, from female cats treated by surgery only. CK5/6 (clone D5/16 B4), CK14 (clone LL002), ER (clone C311), PR (clone 10A9), and HER2 (clone 4B5) expressions were determined by automated immunohistochemistry. The thresholds for positivity were 1% for CK5/6 and 15% for CK14.

Results: CK5/6 positivity was observed in 67/102 (66%) luminal FMCs and associated with better overall survival (HR=0.55), independently of the histological grade (HR=1.75 for Elston & Ellis grade III) and cutaneous ulceration (HR=0.47 if absent) (p=0.0001; Cox proportional hazards regression). CK14 positivity was observed in 197/248 (79%) triple negative FMCs and associated with a higher probability of cancer-related death (HR=1.52), independently of lymphovascular invasion (HR=0.60 if absent) and clinical stage (HR=1.65 for stage 3, 4.68 for stage 4) (p=0.0001).

Conclusions: The "basal" phenotype of feline invasive mammary carcinomas is best defined by CK5/6 in luminal FMCs, and by CK14 in triple negative FMCs. As in human breast cancer, basal cytokeratin positivity worsens the prognosis of triple negative FMCs.

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3

CANINE SPINDLE CELL MAMMARY TUMORS: DIAGNOSIS, GRADING AND CLINICAL BEHAVIOUR

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[*]Dept. Animal Medicine, Surgery and Pathology, Veterinary Medicine School, Complutense University of Madrid (UCM), Spain, [†]Animal and Veterinary Research Centre (CECAV), University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal, [‡]DIVET, University of Milano, Italy and [**]DIMEVET, University of Bologna, Ozzano dell'Emilia, Italy

Introduction: Canine spindle cell mammary tumors (CSCMTs) represent 0.64% of all mammary tumors and their characteristics and clinical behavior are mostly unknown. The aims of the study were to characterize microscopic features, clinical aspects and biological behavior in a series of CSCMT.

Materials and Methods: A total of 70 CSCMTs with demonstrated mammary origin were included in the study. Histology and IHC expression (in 58 cases) of CKAE1/AE3, CK14, vimentin, calponin, p63 and CD31 were evaluated. CSCMTs were graded as sarcomas (s-grade) and as mammary neoplasias (m-grade) when possible.

Results: Dogs with SCMT were of different breeds, generally intact (spayed 25%), with a mean age of 10.7 years. CSCMT were large in size (mean 6.8cm, 53.1% T3) with infrequent lymph node (6.9%) and distant (3.1%) metastases at diagnosis. Lymph node metastases were associated with a high mitotic index (p=0.07) and m-grade III (p=0.011). After IHC, the most common diagnoses were malignant myoepithelioma (MM) (59.3%), carcinoma and MM (16.9%), hemangiosarcoma (8.5%) and PWT/PNST (6.8%). MM, best marked by calponin, were frequently solitary tumors (p=0.049). CSCMTs had low recurrence/metastastatic rate (27.6%). Distant metastases were associated with extensive necrosis (>50%) in the primary tumor (p=0.025) and the histological type of CSCMT (p=0.04). Patients' death cause was generally attributed to development of other non-CSCMT malignant mammary tumors (p= 0.023).

Conclusions: CSCMTs generally demonstrated low recurrent/metastatic potential. Extension of necrosis and identification of the specific histological type with the aid of IHC may assist in the identification of CSCMTs with malignant behavior.

NOTES

4

ANTICANCER SONODYNAMIC TREATMENT WITH PORPHYRIN COMPOUNDS: INSIGHTS ON *IN VIVO* EFFICACY IN A SYNGENEIC RAT MODEL OF CANCER

Capucchio MT., Biasibetti E., Biasato I., Barbero P., Bruni I., Spuria L., Mioletti S., Foglietta F*., Querio G.*, Durando G.#,Canaparo R.*, Serpe L.*

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Introduction: Sonodynamic therapy is an innovative anticancer approach based on the synergistic effect of ultrasound and chemical compound referred to as "sonosensitizer". Aim of the study was to investigate the *in vivo* response of the sonodynamic treatment with ultrasound (US) and the natural porphyrin precursor, 5-aminolevulinic acid (ALA) in a syngeneic rat model of solid mammary tumor.

Materials and Methods: The effects were evaluated in 36 Fisher 344 rats subcutaneously implanted with $1x10^6$ MAT B III cells. 8 days post-inoculum, 24 rats were treated with: ALA (IV, 375 mg/kg) - 5 rats; ALA and US (1.5 Wcm^{-2} at 1.8 MHz for 5 min) - 12 rats, and US only - 7 rats. 12 rats were used as controls. At day 11, all rats were sacrificed and the tumor masses removed and submitted for histological, immunohistochemical (Ki67 and caspase 3) and biomolecular (cleavage of PARP and LC3A/B expression by western blot analysis) investigations.

Results: All the tumors were characterized by a high mitotic index and a variable degree of apoptosis. Voluminous scattered necrotic areas were present. Immunohistochemical investigations showed a decrease of apoptotic cells in all treated tumors. Ki67 expression revealed a severe decrease of mitosis in treated tumors, but particularly in the masses treated by ALA and US. Moreover, a significant expression of the LC3A/B protein was observed in the sonodynamic treated tumors.

Conclusions: Preliminary data suggest a potential therapeutic effect of ALA and US, even if additional investigations are in progress to confirm this hypothesis.

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5

SLAUGHTERHOUSE SURVEY FOR PREVALENCE OF INTESTINAL NEOPLASMS IN RUMINANTS. A COMPARATIVE STUDY

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Introduction: Knowledge of the prevalence of intestinal neoplasms in slaughterhouse surveys in countries where there are few or no available data seems a means of providing data on the interaction of genetic and environmental factors in the etiology of these neoplasms which are frequently diagnosed in human and contribute to the knowledge of comparative oncology. The aim of this study is to acquire objective data on the findings of intestinal neoplasm prevalence in slaughtered ruminants from northern Spain and their histopathological characterization.

Materials and Methods: Young and adult goats (n=9,749), cattle (n=224,941) and sheep (n=788,125) were inspected over a 7-year period in an abattoir in northern Spain. Gross diagnosis was conducted by veterinary inspectors. Tissue samples were collected for their histopathological diagnosis.

Results: Intestinal neoplasms were only detected in sheep (n=34; 0.0043%) and cattle (n=1; 0.0004%) during veterinary inspection. All sheep cases were found in adults (0.072% of adults) and their histopathological examination confirmed 22 adenocarcinomas (91.67% of the neoplasms) and 2 lymphosarcomas (8.33% of the tumours) located in the small intestine except for a colonic mucinous carcinoma. A disseminated abdominal and thoracic neoplasia was observed in gross examination of a cow and its histopathological diagnosis was compatible with metastatic intestinal adenocarcinoma.

Conclusions: Our study revealed an unexpectedly quite high prevalence of sheep intestinal neoplasms in northern Spain, which could increase if an accurate pathological study was conducted in more slaughterhouses. Knowledge of these data could be interesting for veterinary inspectors for differential diagnoses with other diseases.

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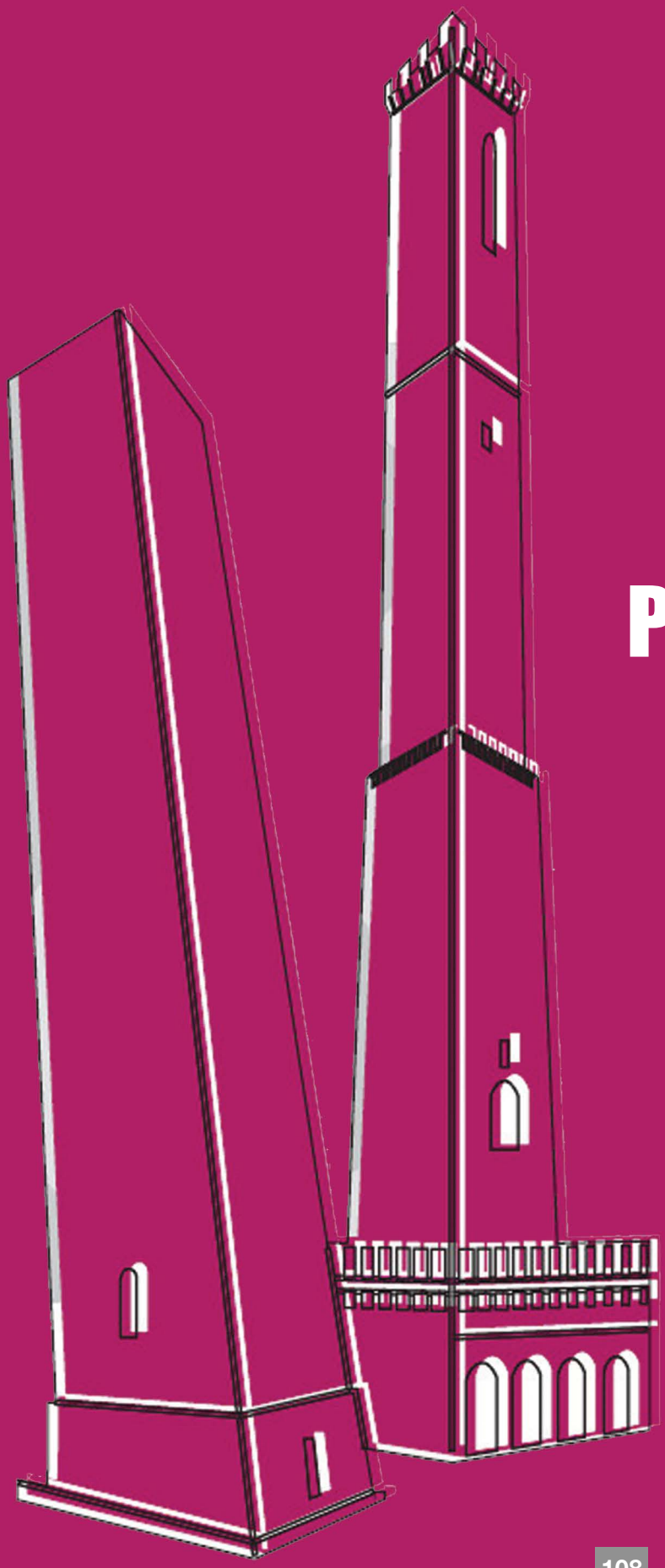

POSTERS FLASH

Session 2 - Oncology

6

BRACKEN AND PAPILLOMAVIRUS-INDUCED CANCERS: WHICH TOXIN IS THE MAIN VIRAL CO-FACTOR?

R.M. Gil da Costa[*§], S. Aragão[†], M. Moutinho[†], A. Alvarado[†], T. Neto[†‡], C. Santos[‡], P. Ferreirinha[‡], D. Carmo[£], F. Casaca[£], S. Silva[£], J, Ribeiro[§], H. Sousa[§], R. Medeiros[§‡], C. Lopes[§‡], M. Vilanova[‡], P. A. Oliveira[†‡], M. Bastos[*]

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Introduction: Bracken toxins like ptaquiloside and quercetin are thought to drive the progression of upper digestive cancers initiated by bovine papillomavirus type 4 (BPV4), and bladder cancers related with BPV1/2. We used human papillomavirus type 16-transgenic (K14-HPV16) mice to study the effects of ptaquiloside and quercetin on carcinogenesis and the effects of ptaquiloside on tumour-infiltrating $CD8^+$ T lymphocytes.

Materials and Methods: Female K14-HPV16 mice were orally administered ptaquiloside (10 weeks at 0.5mg/mouse/week) or rutin (quercetin rutinoside, 70.0mg/mouse/week for 15 weeks) and euthanized at 30 weeks-old. Skin, bladder and whole heads were processed histologically. Cutaneous tumour-infiltrating $CD8^+$ T cells were analysed for CD107a and CD44 expression using flow cytometry. The study was approved by the Portuguese Veterinary Directorate (approval number 0421/000/000/2014).

Results: At 30-weeks-old, 50% untreated mice showed hard palate squamous cell carcinoma (SCC) and multifocal pre-malignant oral lesions. Quercetin had no effect on lesions incidence or distribution. Ptaquiloside increased SCC incidence in multiple oral sites. No histological bladder lesions were observed in any group. On skin biopsies, 100% of ptaquiloside-treated mice showed diffuse epidermal dysplasia, compared with 50% untreated mice. Ptaquiloside-exposed mice showed reduced (P 0.05) $CD8^+CD107a^+$ and $CD8^+CD44^+$ T cells percentages compared with untreated mice.

Conclusions: K14-HPV16 mice are useful for studying oral carcinogenesis but their usefulness in the context of bladder carcinogenesis requires further studies. Ptaquiloside - rather than quercetin - seems to be the main bracken co-factor involved in oral carcinogenesis in this model. This may involve imunosuppression through decreased $CD8^+$ T cell memory activation and degranulation.

NOTES

7 CHARACTERIZATION OF CANINE SMOOTH MUSCLE TUMOUR: PILOT STUDY ON 68 CASES

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Introduction: Canine smooth muscle tumours (SMTs) usually arise in the gastrointestinal and urogenital tract but rarely in soft tissues. While the criteria used to identify smooth muscle differentiation are well established, the distinction between leiomyomas and leiomyosarcomas are based on mitotic activity, cellular atypia and amount of necrosis, but reliable cut-off values are lacking.

Materials and Methods: Cases of canine SMTs were retrospectively collected and histologically examined. Differentiation, cellular atypia, necrosis amount mitotic count (MC), and MIB1-based labeling-index (LI) were assessed and statistically analyzed.

Results: Sixty-eight SMT were collected in 67 dogs (M/F=0.5). Twenty-seven SMTs were uterine/vaginal, 18 gastrointestinal, 10 soft tissue, 9 urinary, and 4 splenic. Twenty-five SMTs were leiomyomas and 25 leiomyosarcomas. Eighteen SMTs were not classifiable based on veterinary criteria and were diagnosed as SMTs with uncertain malignant potential according to human medicine criteria. MC ranged between 0 and 59 (mean 13.6; median 4), and LI between 0 and 30.1 (mean 14.6; median 4.7). Diagnosis was significantly associated with gender ($p<0.01$), being leiomyomas more frequent in female and leiomyosarcomas in male, and with LI ($p<0.01$), higher than 5 in 72% of leiomyosarcomas and ≤ 5 in 76% of leiomyomas.

Conclusions: Relying on veterinary criteria, the differential diagnosis between leiomyomas and leiomyosarcomas was not possible in 18 cases that, despite well-differentiated, had a slightly higher MC than leiomyomas and some degree of cellular atypia and necrosis. We suggest to use LI with a cut-off of 5 to distinguish leiomyomas from leiomyosarcomas when histology alone is not sufficient.

NOTES

8

17-AAG INHIBITS VASCULOGENIC MIMICRY OF METASTATIC OSTEOSARCOMA CELL LINE

M. Massimini, A. D'Anselmo, D. Malatesta, M. Romanucci, L. Bongiovanni, L. Della Salda

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Introduction: Tumour vasculature is derived from a variety of sources including vasculogenic mimicry (VM). Treatments based on Hsp90 (Heat-shock-protein-90) inhibition negatively influences several factors involved in VM regulation including VegfR1 and Hif1α. Aim of the study was to verify the ability of two canine osteosarcoma cell lines to generate VM in 3D culture, identify specific markers of endothelial-like cells and estabilish 17-AAG (17-N-allyloamino-17-demethoxygeldanamycin) activity on tubular-like structure formation *in vitro* and degradation of Hsp90 client proteins.

Materials and Methods: D22 and D17 canine osteosarcoma cells were seeded on Collagen Rat Tail Type 1 and the presence of tubular-like structures was evaluated. After 4 weeks, sections of cultures were H&E stained or exposed to immunohistochemical evaluation of CD31, vWf, VegfR1 and HSp90. Cells were also treated with 17-AAG and principal VM features, cells migration and VegfR1 and Hif1α were quantified.

Results: Only metastatic cells showed tubular-like structures in 3D culture. Histological investigation of D17 3D long term culture further demonstrated the presence of vessel-like channels showing cavities in serial transversal sections surrounded by endothelial-like cells expressing Hsp90 and VegfR1. Furthermore, 17-AAG induced a significant decrease of VM features in a time-dependent manner.

Conclusions: 17-AAG activity on canine osteosarcoma 3D culture could offer new prospects for the development of therapeutic strategies based on the synergy between VM-blocking and anti-angiogenic property determined by hsp90 inhibition. This is the first study that highlight the presence of vessel-like structure in long term canine osteosarcoma 3D cultures, laying the technical groundwork to search specific tumour endothelial-like cell markers.

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9

ATYPICAL HISTOCYTIC-LIKE MAST CELL TUMOURS IN HORSES

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Introduction: Cutaneous mast cell tumours (MCT) are frequent neoplasms in domestic animals, where different morphological variants are reported. In horses, they are uncommon and most frequently present as a proliferation of well differentiated mast cells with associated eosinophilic granuloma (EG)-like lesions. Here we report on a group of four lesions in horses with morphological features consistent with a histiocytic variant of MCT.

Materials and Methods: As part of a larger study we reassessed a population of equine MCT and EG (191 cases), applying histochemistry (Toloudine blue stain) and immunohistochemistry (CD117, PCNA, mast cell tryptase, and lysozyme).

Results: Four lesions (two each conjunctival and dermal), initially diagnosed as EG-like processes, formed a distinct group. They were composed of medium sized to large pleomorphic round cells with a large central nucleus and abundant cytoplasm, embedded in large numbers of mature eosinophils. The largest cells were identified as atypical mast cells, while the medium sized cells showed a histiocytic immuo-phenotype. In two cases, atypical mast cells exhibited aberrant CD117 expression but a variable degree of proliferation (i.e. PCNA expression). The histiocytic appearance upon haematoxylin eosin of the large atypical mast cells prompted us to classify the lesions as “histiocytic-like MCT”.

Conclusions: In cats, a histiocytic mast cell tumour variant is an established entity, the diagnosis of which is based solely on the morphology of the neoplastic cells. Applying some of those criteria, we identified an equine equivalent. Interestingly, this variant seems to frequently exhibit an aberrant CD117 expression pattern.

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RABBIT TESTICULAR SEMINOMA: A HISTOCHEMICAL AND IMMUNOHISTOCHEMICAL STUDY

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Introduction: In rabbits spontaneous seminomas are reported, but few data about their incidence, clinical behaviour and immunohistochemical profile are present, even if they could represent a model for human counterpart. Human WHO classification of testicular tumours includes two types of seminoma: spermatocytic seminoma (SS), originating from the post-pubertal spermatogonia/spermatocytes, and classical seminoma (SE), originating from fetal gonocytes, considered malignant and metastasizing. Gonocytes are recognized by periodic acid-Schiff (PAS) staining and by placental-like alkaline phosphatase (PLAP) expression.

Materials and Methods: To evaluate analogies with the human counterpart, immunohistochemistry for PLAP and PAS staining have been performed on five cases of seminoma in pet rabbits, ranging in age from 5-9 years.

Results: all cases were diffuse seminoma with multifocal/diffuse necrotic areas, severe anisocytosis and anisokaryosis and high mitotic count. One case metastasized to sublumbar lymph node. PLAP staining was diffuse and intense in this latter case, while in 2/5 cases was multifocal, in 1/5 case was limited to rare neoplastic cells and 1/5 case was negative. PAS+ neoplastic cells were detected exclusively in the 4/5 PLAP+ cases. These four cases were therefore classified as SE, while the negative one was considered SS.

Conclusions: Results suggest that in rabbit, as in men, two different seminoma exist: SS and SE. SE seems to be well represented, including a metastasizing case. These findings evidence analogies with the human counterpart and suggest further studies on a larger number of samples to evaluate rabbit as possible animal model for the study of human seminoma.

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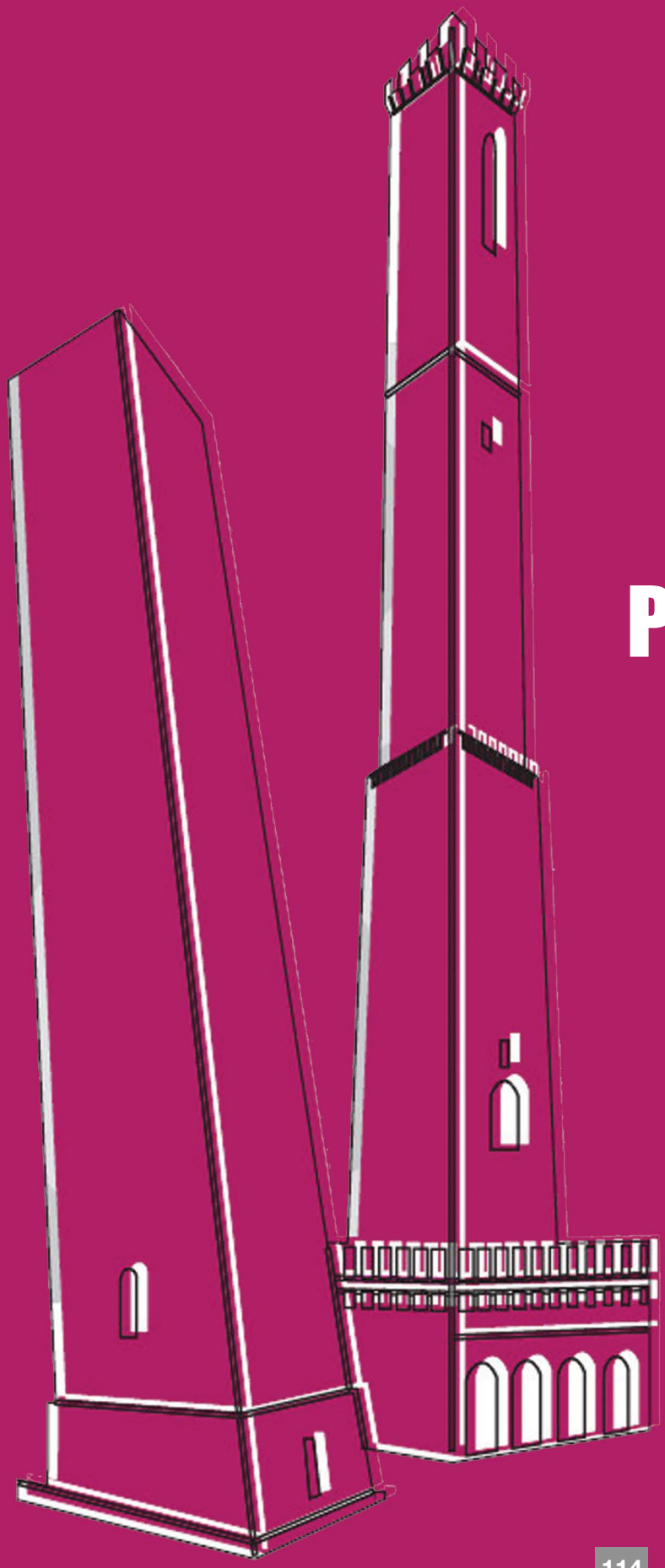

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Session 3 - Neuropathology

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NEUROPATHOLOGICAL PHENOTYPE OF *L. MONOCYTOGENES* LINEAGE I AND II INFECTIONS

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Introduction: In farmed ruminants, *Listeria (L.) monocytogenes* infection is mainly associated with neurolisteriosis and typically manifests as brainstem infection (rhombencephalitis). *L. monocytogenes* is a genetically diverse species divided into two major (I and II) and two minor (II and IV) phylogenetic lineages. Although isolates from ruminant rhombencephalitis belong to both major phylogenetic lineages, lineage I strains are strongly overrepresented in rhombencephalitis. The aim of this study was to compare the neuropathological phenotype of rhombencephalitis in lineage I and lineage II infections.

Materials and Methods: The neuropathology of naturally occurring rhombencephalitis cases in small ruminants was compared on H&E sections of representative brain regions including brainstem, cerebellum, midbrain, thalamus, basal nuclei and cortex. Additionally, IHC for *L. monocytogenes* was performed and the bacterial load was assessed. Fifty-one cases of lineage I infection and 27 cases of lineage II infection were assessed.

Results: Lesions were similar between infection with lineage I and II, and no discriminating neuropathological feature was identified. However, some neuropathological changes including axonal, vascular and neuronal necrosis were more prevalent in infections with lineage I. Additionally, lineage I infections had a higher bacterial load in chronic lesions, and associated lesions spread further rostrally in the brain than in lineage II infections.

Conclusions: Our results suggest a more efficient intraphagocytic persistence of *L. monocytogenes* lineage I strains and corroborate their hypervirulence as indicated by epidemiological studies and experimental infections in cell lines and mice.

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NF-kB mRNA UP-REGULATION AND INHIBITION OF APOPTOSIS IN THE BRAINS OF TOXOPLASMA GONDII INFECTED MICE

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Introduction: Tissue cysts of *Toxoplasma gondii* localize in the host brain and inhibition of apoptosis in a host cell infected by *Toxoplasma gondii* Tip II strain seems in favor of the parasite survival. Up to date, there is no data on the NFκB activity, related gene expressions and anti-apoptotic pathway in the central nervous system of *T. gondii* infected animals.

Materials and Methods: An experimental chronic encephalitic toxoplasmosis model was established using *T. gondii* type II strain in mice. The aim was to investigate NFκB activity and related mRNA expressions of pro-inflammatory cytokines (IL1, TNF-α) and apoptotic markers (caspase 3, caspase 9) using quantitative Real Time PCR at 10, 20 and 30 days after inoculation.

Results: NFκB activity was prominently higher in *T. gondii* infected mice brains on DAI 10, 20 and 30 than control healthy mice ($p<0.05$). IL1 and TNF-α mRNA, expressions showed an important increase starting from DAI 20 in *T. gondii* infected mice ($p<0.05$). Caspase 3 was down-regulated on DAI 10 and 20 in the brains of *T. gondii* infected mice ($p<0.05$).

Conclusions: In conclusion, it is established that NFκB activity originated from the *T. gondii* infected cells has been contributing to the anti-apoptotic pathway and this is one of the most important mechanisms allowing the tissue cysts' lifelong permanence in the host central nervous system. The issue on the sources of NFκB in toxoplasmosis or which cells can contribute to NFκB pathway is the subject of future neural cell culture studies.

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NEUROPATHOLOGICAL FINDINGS IN EQUIDS DURING WEST NILE DISEASE SURVEILLANCE ACTIVITIES IN PIEDMONT, ITALY

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Introduction: West Nile (WN) is a widespread disease caused by an arthropod-borne virus belonging to the genus Flavivirus. In the Mediterranean basin it is mainly transmitted by Culex mosquitoes. In Italy from 1998 to 2015 a total of 170 and 191 neurological cases in humans and horses respectively have been reported. Complying with Italian sanitary law, equids dying with neurological signs must be submitted to neuropathological examination to exclude WN virus infection.

Materials and Methods: From 2002 to 2015 during WN surveillance activities, 37 equids dying with neurological signs were sent to Istituto Zooprofilattico Sperimentale of Turin for neuropathological investigation. The Central Nervous System was collected and histological and molecular examinations were performed.

Results: Neuropathological changes were visible in 8 cases: 4 inflammatory lesions, 1 neoplasia, 1 parasitic disease, 1 vascular and 1 metabolic-toxic encephalopathy. In 3 horses brain showed non specific lesions. No histological lesions were found in 13 of tested samples and a further 13 cases were unsuitable for evaluation. No cases of WN disease were diagnosed.

Conclusions: In Italy there is currently in place a WN monitoring plan that obligates to test blood and organs of every equids with neurological signs to exclude the virus infection. Surveillance Systems are a public services based on specific activities to early detect important pathogens in the Country, allowing the collection of useful information for diseases control and to assess their impact on the population. Neurological disorders in equids are probably underestimated and the WN surveillance could help to detect cases that would be otherwise lost.

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APOPTOSIS RESISTANCE OF LYMPHOCYTES IS ASSOCIATED WITH PROGRESSIVE DEMYELINATION IN A MURINE MODEL FOR MULTIPLE SCLEROSIS

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Introduction: Theiler's murine encephalomyelitis (TME) is induced by the TME virus (TMEV) and characterized by acute polioencephalomyelitis and chronic demyelinating leukoencephalomyelitis. Due to morphological and pathogenic similarities TME represents an important animal model for human multiple sclerosis (MS). Oligodendroglial apoptosis seems to participate in autoimmune demyelination in MS. Furthermore, reduced apoptotic elimination of autoaggressive leukocytes might maintain persistent inflammation in advanced MS lesions. However, the role of apoptosis in the TME demyelination process is still under debate.

Materials and Methods: Apoptotic cells were quantified in the spinal cord of SJL mice infected with the BeAn strain of TMEV using TUNEL staining and immunohistochemistry for active caspase-3. Electron microscopy and double-immunofluorescence were used for further characterization of apoptotic events. Transcriptional changes of apoptosis-related genes were investigated using microarray analysis.

Results: Highest apoptotic cell numbers were found at 42 days post infection (dpi), followed by a decrease during the late disease phase (98 and 196 dpi). Microarray analysis detected the induction of classical pathways of apoptosis as well as alternative mechanisms of cell death such as pyroptosis and endoplasmatic reticulum stress in spinal cord lesions. Oligodendroglial apoptosis was already detected at 14 dpi preceding demyelination, whereas apoptotic lymphocytes were hardly present at 196 dpi.

Conclusions: Oligodendroglial apoptosis seems to play a prominent role in the initiation and lymphocyte apoptosis resistance in the progression of the TME demyelination process. Results might represent the basis for new therapeutic concepts in demyelinating CNS diseases including MS.

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Session 4 - Infectious diseases

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BLASTOCYSTIS SP.: A NEW DIARRHEAL PATHOGEN?

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Introduction: *Blastocystis* sp. is a cosmopolitan and ubiquitous protozoal parasite which is frequently found in the intestine of humans and animals, such as pigs and cattle. Although *Blastocystis* was first described in 1911, pathogenesis and pathogenicity are still not fully understood.

Materials and Methods: In March 2015, a putative case of blastocystosis occurred in a piglet producing farm in Austria. Except for fulminant diarrhea and wasting, no other clinical symptoms could be observed. Because of the declined state of health and resistance to therapy, one piglet was euthanized for necropsy. Tissue samples were obtained for pathohistological examination and further investigations, including PCR and in- situ hybridization (ISH). To exclude PCV-2 associated enteritis, ISH was carried out on FFPE gut tissue samples. Feces from the large intestine were subjected to a triplex PCR for simultaneous detection of *Lawsonia intracellularis*, *Brachyspira hyodysenteriae* and *Brachyspira pilosicoli*. For identification of coccidian oocysts and protozoal parasites, fluorescence microscopy and conventional light microscopy of feces were conducted.

Results: Histologically, gut tissue samples showed physiological architecture and no pathological alterations could be observed. In close proximity to epithelial cells and within luminal material, numerous vacuolar forms of *Blastocystis*, with no evidence of attachment or invasion, could be discovered. In addition, neither molecular methods nor bacteriological and parasitological methods identified any other pathogenic agent.

Conclusions: The fact that in all investigated samples none of the common enteropathogenic agents in piglets could be found, suggests a major etiological role of the massive *Blastocystis* sp. infestation.

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EPIDEMIOLOGICAL STUDIES ON BIOLOGICAL AGENTS IN MACROSCOPICALLY HEALTHY SMALL RUMINANT MAMMARY GLANDS

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Introduction: The importance of small dairy ruminants has increased, especially because they represent an important alternative to supply dairy products for human consumption. A microbiological and pathological study was carried out to evaluate the biological agents isolated from macroscopically healthy udders of regularly slaughtered small ruminants correlating their presence with the histological features observed.

Materials and Methods: Eighty-nine macroscopically healthy udders of small ruminants were randomly collected between October 2013 and February 2016 in Piedmont region (North of Italy). After the macroscopical evaluation one fragment of parenchyma was collected, fixed in 10% formalin for histopathological examination, the remaining tissue was used to perform bacteriological, virological and mycological investigations. Multinomial logistic regression was applied to evaluate the association among lesions and positivity to different isolates, and bacteria.

Results: Twenty-five samples were microbiologically negative; in the positive udders, 138 different bacterial species were isolated. Coagulase-negative staphylococci-CNS were the most prevalent bacteria isolated (46.42%), followed by environmental opportunists (34.76%), other (10.14%) and pathogens (8.68%). Histologically the absence of lesions were observed in 28 samples, the remaining 61 udders showed different types of mastitis: chronic non suppurative mastitis (50.56%), chronic mixed mastitis (13.48%) and acute suppurative mastitis (4.5%). Lentivirus infection was present in 39.3% of samples. Histological lesions were significantly associated to small ruminant species and Lentivirus and CNS infections.

Conclusions: The results suggest that unaffected glands of small ruminants are a reservoir of biological agent. This information can be a help to improve hygiene and quality of dairy products, and consequently consumers' welfare.

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ZINC OXIDE NANOPARTICLES AS A NOVEL TOOL TO COMBAT *YERSINIA RUCKERI* AND APHANOMYCES INVADANS

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Introduction: *Yersinia ruckeri* is the causative agent for enteric red mouth disease (ERM) in fish. This disease has a wide host range, broad geographical distribution and results in significant economic losses in fish farms. In spite of the production of a vaccine against *Y. ruckeri*, incidence of vaccination failure for non motile strains had been reported. Epizootic ulcerative syndrome (EUS) outbreaks caused by *Aphanomyces invadans* induce skin ulceration which extends deeply to the underlying muscles leading to morbidity and mortality in fish. The intensification in aquaculture activities worldwide led to massive usage of antibiotics with rising of microbial resistance problem. Genes of antibiotic resistance can be transferred from aquatic bacteria to animal and human bacteria, which impose a great hazard on human public health. From this point arise the need for alternative antibacterial agents to combat fish pathogens.

Material and methods: In this study, we investigated the antimicrobial effects of zinc oxide nanoparticles (≈ 66 nm) against *Y. ruckeri* and *A. invadans in vitro*. Characterization of nanoparticles was performed using zeta sizer and electron microscopy. Minimal inhibitory concentration (MIC) for both *Y. ruckeri* and *A. invadans* were determined.

Results: *Y. ruckeri* growth was inhibited after incubation with zinc oxide nanoparticles at 31.5 µg/mL concentration while it was found that 3.15 µg/mL of zinc oxide nanoparticles is capable to inhibit growth of *A. invadans*

Conclusion: This is the first record that zinc oxide nanoparticles exhibit efficient antimicrobial activity against *Y. ruckeri* and *A. invadans*.

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COMPARATIVE STUDY OF DIAGNOSTIC TECHNIQUES FOR DETECTION OF EARLY INFECTION WITH TOXOPLASMA IN PIGS

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Introduction: *Toxoplasma gondii* is an important zoonotic agent. Ingestion of undercooked pork is one of the main sources of infection. Several diagnostic tools have been developed for detecting this parasite in pig tissues. In this study the diagnostic value of histopathology, immunohistochemistry, real time PCR (rtPCR) and murine and feline bioassays was evaluated in pigs experimentally infected with different doses of *T. gondii* strain TgH00001.

Materials and Methods: Eleven pigs were used: Group-1 (n=5), IM infected with 10^7 tachyzoites; Group-2 (n=4), IM infected with 10^3 tachyzoites and Group-3 (n=2), negative control. Blood was collected at 0, 15 and 30 dpi and analysed by ELISA. At 30 dpi all the animals were euthanised and samples from meat, brain, heart, tongue, masseter muscle, lungs, liver, kidney, spleen and mesenteric lymph node were collected and studied by rtPCR, histopathology and immunohistochemistry. Meat was also studied by feline bioassays and pools of target tissues (brain, heart, tongue) by murine bioassays.

Results: An increase in seropositivity was observed, but always below the cut-off. rtPCR from meat and brain showed significant differences between groups (positive in 4/5 animals from Group 1 vs. 1/4 and 0/4 from Group 2). Heart, masseter muscle, mesenteric lymph node and kidney samples were more sensitive (8/9). Feline and murine bioassays were only positive at high doses. Histopathology failed to detect *T. gondii*. Immunohistochemistry was inconsistently positive.

Conclusions: Our results underline the importance of infective dosage in parasite distribution and selection of target tissues for the diagnosis, with rtPCR as the most sensitive tool.

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DETECTION OF DOG CIRCOVIRUS IN A DOG WITHOUT TYPICAL LESIONS – WHAT DO WE KNOW ABOUT SUBCLINICAL INFECTION?

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Introduction: Dog Circovirus (DogCV) has been described in dogs in the USA and Italy and is associated with vasculitis, hemorrhage, lymphadenitis/lymphadenopathy, and hemorrhagic gastroenteritis. Concurrent pathogens are often detected. A closely related circovirus was recently detected in foxes with meningoencephalitis in the UK. The pathogenesis of DogCV infection is incompletely understood.

Materials and Methods: Post mortem tissue samples from 52 dogs were analyzed using a specific PCR approach, and other common canine pathogens were excluded by PCR or microbiology. All organs were evaluated using routine histopathological stainings. The detected virus strain was completely sequenced.

Results: DogCV was detected in the liver and spleen of one dog that had died of a histiocytic sarcoma, apparently not correlated with the viral infection. The detected strain (acc. no. KT283604) is closely related to strains UCD-1, UCD-2 and Bari, thus grouped in “cluster 1” of DogCV.

Conclusions: This is the first described case of DogCV infection in the Berlin area, pointing towards an increasing importance of this virus in Central Europe. DogCV was detected in parenchymal organs in the absence of any obvious associated lesions, possibly suggesting previous infection with persistence. Subclinically infected dogs may be important for the spread of the virus. Future studies need to focus on the actual prevalence, epidemiology and spread of the virus and investigate the conditions that lead to clinical disease. This study also provides specific tools for retrospective analyses.

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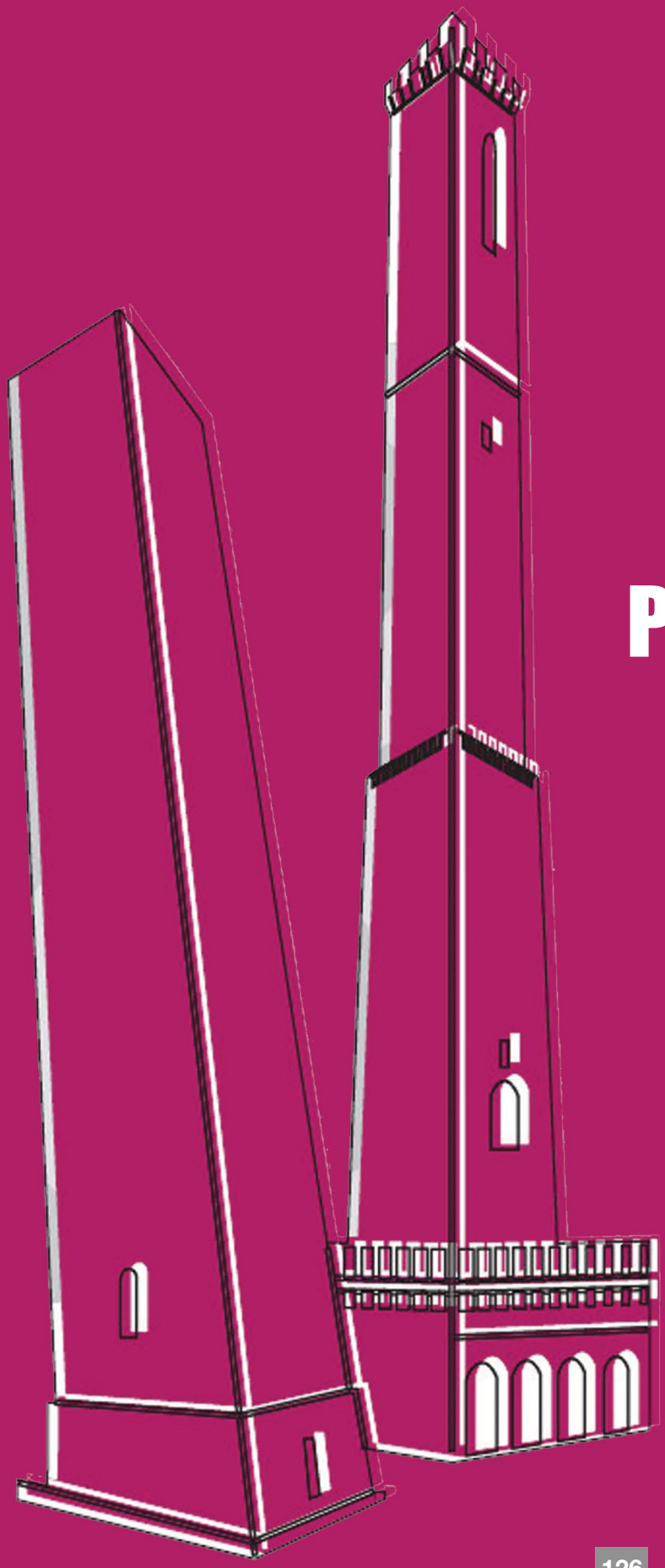

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Session 5 - Miscellaneous

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GROWTH PLATE LESIONS OF 62 FATTENING BULLS AT THE ABATTOIR IN THE NORTH-EAST OF ITALY

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Introduction: Lameness caused by growth plate lesions is a problem in beef cattle, however there are no recent reports on this topic. This paper describes the macroscopic and microscopic lesions of the distal metatarsal physis of bulls from farms in North-Eastern Italy.

Materials and Methods: The distal metatarsal physes from 62 bulls of 16.44 ± 1.72 months of age were examined; 12 bulls were slaughtered due to severe lameness (Group A), and 50 were slaughtered with no signs of lameness (Group B). Animals were from the same area and shared similar intensive husbandry practices and diet. Grossly, 124 metatarsi were examined. Four distal metatarsal bones from grossly normal bulls, 24 from Group A and 18 from Group B with macroscopic lesions, were examined microscopically.

Results: Macroscopic lesions present in Group A bulls included 1 bilateral purulent chronic physitis (PCP), 7 unilateral PCP, 3 bilateral physeal osteochondrosis (OCD), 6 unilateral OCD, and 3 unilateral purulent tenosynovitis. Nineteen bulls (38%) from Group B also presented with macroscopic OCD lesions (13 bilateral and 6 unilateral), confirmed by histology in 6 cases. On microscopic examination severe segmental thickening of the hypertrophic zone was present, consistent with physeal OCD. In animals with PCP, the physis was necrotic with eosinophilic cartilage matrix, extensive neutrophilic infiltrate, hemorrhage, and fibrin deposition.

Conclusions: A high incidence of OCD was found in lame and non-lame fattening bulls. Hematogenous bacterial implantation in the metaphysis led to a purulent physitis that resulted in severe lameness and necessitated emergency slaughtering of affected animals.

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DISTURBED NEUROGENESIS IN A TOXIC MODEL FOR HIPPOCAMPAL DEGENERATION

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Introduction: Experimental feeding of the copper chelator cuprizone causes neuronal loss in the murine hippocampus and leads to seizures in treated animals. Aim of this study was to investigate the impact of hippocampal damage upon the neurogenesis within the dentate gyrus.

Materials and Methods: Neurogenesis in the dentate gyrus of experimentally cuprizone-fed mice and control animals neurogenesis was quantified by doublecortin (DCX)- and Ki-67-immunohistochemistry. In addition, astrogliosis and apoptosis were measured by glial fibrillary acidic protein (GFAP)-staining and TUNEL-method. The effect of cuprizone upon differentiation and viability of NT2 cells as well as lactate dehydrogenase (LDH) release of two murine neuroblastoma cell lines was investigated *in vitro*.

Results: A significant reduction of the neurogenesis (DCX) and proliferation of neurons (Ki-67) within the dentate gyrus of cuprizone-fed mice was detected. This was associated with a significant increase of astrogliosis (GFAP) and apoptosis (TUNEL) in treated mice compared to non-treated mice. These findings were reversible after removing cuprizone from the diet. There was no detectable effect of cuprizone upon the neurogenesis and viability of NT2 cells and the amount of LDH in the supernatant of neuroblastoma cell lines.

Conclusions: Results show an inhibitory effect of cuprizone upon the hippocampal neurogenesis, which is reversible and probably mediated by indirect mechanisms.

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23 COMPARATIVE ASSESSMENT OF THE ACCURACY OF CYTOLOGICAL AND HISTOLOGICAL BIOPSIES IN THE DIAGNOSIS OF CANINE BONE LESIONS

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Introduction: Primary bone tumours account for 2-7% of all canine malignancies and are mostly represented by osteosarcoma. Osteosarcomas should be differentiated from other less frequent neoplasms, metastatic disease and tumour-like lesions, because therapy and prognosis can vary accordingly. Hence, a histological diagnosis is generally preferred. This requires collection of multiple biopsies under general anaesthesia, with possible complications, including increased pain and pathological fractures. Fine-needle aspiration cytology (FNAC) would allow an earlier diagnosis with a significant reduction of patient discomfort.

Materials and Methods: The accuracy of FNAC and histological biopsy (HB) in the diagnosis of canine osteolytic bone lesions was assessed by comparing the former diagnosis with the final histological diagnosis on surgical or post-mortem samples or, in the case of non-neoplastic lesions, with follow-up information.

Results: The study included 50 malignant primary tumours (40 osteosarcomas, 5 chondrosarcomas, 2 fibrosarcomas and 3 poorly-differentiated sarcomas), 6 carcinoma metastases and 12 non-neoplastic lesions. Accuracy was 84.9% for FNAC (sensitivity, 83.3%; specificity, 80%) and 82.1% for HB (sensitivity, 72.2%; specificity, 100%). Tumour type was correctly identified in 60% and 55.5% of cases, respectively.

Conclusions: The accuracy of FNAC was comparable to HB and in no case was a benign lesion diagnosed as malignant. This is the most important error to prevent, as therapy for malignant bone tumours includes demolitive surgery. Both methods were also similar in the determination of tumour type. Being a reliable diagnostic technique, cytology should be further considered to aid decisions in the preoperative setting of canine bone lesions.

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OCCIPITAL CONDYLAR DYSPLASIA IN A JACOB SHEEP

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Introduction: Jacob sheep (*Ovis aries*) are a popular pedigree breed, known for their "polycerate" (multihorned) phenotype. This trait has been associated with occipital condylar dysplasia of varying severity in a small number of cases in the USA. In these cases, ataxia was the main clinical sign observed, and the affected lambs ranged in age from new born to four months.

Materials and Methods: A pedigree, four-horned male Jacob lamb presented with progressive congenital hindlimb ataxia, which worsened until difficulty rising from recumbency was frequently observed. It remained bright and alert, and had no difficulty feeding. The lamb was euthanased at four weeks of age.

Results: Gross examination revealed marked deformity, asymmetry and lateral deviation of the occipital condyles with narrowing of the foramen magnum. The spinal cord was grossly compressed at that level and marked axonal degeneration was confirmed by histology.

Conclusions: These findings are consistent with severe occipital condylar dysplasia. This is the first reported case in European-bred Jacob sheep. Occipital condylar dysplasia should be considered as a differential diagnosis in four-horned Jacob lambs showing ataxia. It is important to raise awareness of this disease due to its suspected heritable nature and purported link to the polycerate trait, which is increasingly popular.

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MARE OVARIECTOMIES FOR "POLYCYSTIC OVARIAN DISEASE"

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Introduction: Ovariectomy is a common treatment for "behavioral problems" of mares. Owners and veterinarians use the term "stallion-like" to describe many untoward behavior in mares. Many cases of ovariectomy have no neoplasms or hemorrhagic anovulatory follicles and although the behavior disappears, pathologists and clinicians have no diagnosis. Polycystic ovarian syndrome in women, including young athletes, is characterized by a "polycystic" ovarian ultrasound appearance, hyperandrogenism, gonadotrophin abnormalities, obesity, and insulin resistance.

Materials and Methods: In the last 12 months, cases of behavior problems exacerbated "under saddle" that resulted in bilateral ovariectomies and that did not involve neoplasia or non-anovulatory follicles were reviewed and characterized.

Results: Three cases of 4-8 year-old mares in our service fit the above criteria and included a barrel racer, one dressage mare and one pleasure mare. The mares all had behavior problems suggesting aggression, but questioning revealed that their symptoms actually included behavior under saddle that prevented competition or safe riding, and had an unacceptable response to altrenogest supplementation and had elevated serum testosterone. The mares had multiple, >1-2cm follicles with both growing and atretic follicles, and two had recent ovulations.

Conclusions: Mares may present with cases similar to polycystic ovarian syndrome and/or pelvic pain syndrome of women. These mares are relatively young, cycling athletes. When dealing with these syndromes, we must consider fibrothecomas in our diagnoses. In work-ups we should include body condition scoring, anti-inflammatory response, serum hormone levels, immunohistochemistry and possible blood glucose abnormalities to fully evaluate similarities to the human condition.

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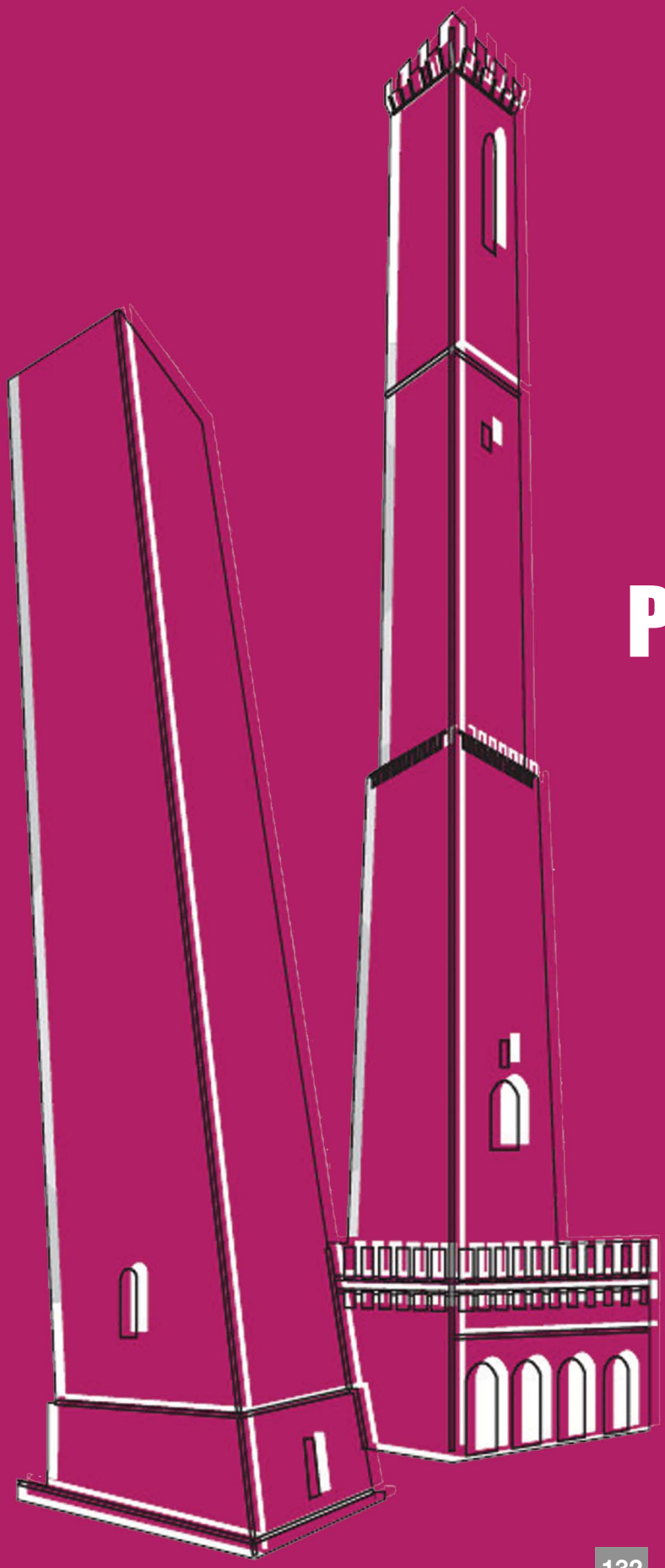

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Session 6 - Miscellaneous

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BIOCOMPATIBILITY STUDIES OF LOCAL ANTIBIOTIC-ELUTING DEVICES FOR ORTHOPEDICS APPLICATIONS

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Introduction: Efficient local antibiotic-eluting devices could be an alternative to deliver locally therapeutic antibiotics at tissues, avoiding bacterial contamination on implanted materials and minimizing side effects. A proper assessment of biocompatibility of the biomaterials used is important to improve safety after implantation. We present cytotoxicological and implantation tests results to evaluate biocompatibility of two drug-eluting systems with potential use in orthopedic implants.

Materials and Methods: Cytotoxicological studies were carried out by evaluating the in-vitro dose-dependent effect of cefazolin and linezolid in fibroblasts, keratinocytes macrophages and osteoblasts. Cells were incubated with antibiotic concentrations ranging from 0.25-1.5 mg/ml. Cellular viability was assessed by the Alamar Blue test. Cell cycle and apoptosis were measured by flow cytometry. Short-term implantation tests were performed in an ovine model to assess the device's local effects. Two implants were used, (A) macroporous stainless steel reservoir loaded with linezolid and (B) stainless steel pins with orifices drilled in the reservoir wall loaded with cefazolin. Implants were placed in sheep's tibia. Tissues were studied by pathological means, determining the local effect and tissue response from the implant. (Ethical committee number PI36/14)

Results: Cytotoxic effects of cefazolin and linezolid were only found at 1.5 mg/ml on keratinocytes and osteoblasts, respectively. There were no significant changes on cell cycle and apoptosis at 1.0mg/ml. Sheep with both A and B antibiotic-loaded implants did not show local or systemic adverse effects.

Conclusions: These results showed no potential toxic effects for the designed devices. However, the antibiotic local concentration should not exceed 1.0 mg/ml.

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SECONDARY ACORN (*QUERCUS SP.*) POISONING IN TWO DOGS

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Introduction: Two unrelated, adult dogs living in the same household showed anorexia, lethargy and vomiting for a few days. The dogs lived in close contact with a pet pig, which was recently fed a large amount of acorns (*Quercus sp.*). The animals had a habit of eating the pig's feces. Both dogs were azotemic and had increased ALT levels. The dogs were diagnosed with acute kidney injury. Despite supportive treatment, they did not improve and were euthanized.

Materials and Methods: At necropsy, FFPE tissue samples were collected and stained with H&E. Liver was collected for pyrogallol (one of the toxic metabolites of acorns) detection.

Results: Both dogs had similar kidney lesions with extensive degeneration, necrosis and multifocal mineralization of the epithelial cells of the proximal tubules. Eosinophilic casts were multifocally present in the tubular lumen. The liver tested positive for pyrogallol.

Conclusions: This is the first reported case of acorn poisoning in dogs and the first reported case of secondary acorn poisoning in any species. Acorn poisoning is mostly seen in cattle, where, as in these cases, acute tubular necrosis is the most prominent lesion. Cattle usually also show multifocal edema, renal hemorrhages and intratubular hemorrhagic casts, which were not seen in these dogs. The observed tubular mineralization, on the other hand, is usually not reported in bovine cases.

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VITAMIN D LEVELS ARE LOWER IN HEALTHY IMMATURE COMMON MARMOSETS (*CALLITHRIX JACCHUS*) THAN IN HEALTHY ADULTS

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Introduction: New world primates such as Common Marmosets (*Callithrix jacchus*) overexpress the vitamin D response element binding protein, which requires them to have higher circulating vitamin D levels compared to other animals. A few authors previously published serum vitamin D and parathyroid hormone (PTH) levels in small numbers of marmosets, observing wide variations for vitamin D, but none compared large groups of immature marmosets to mature animals.

Materials and Methods: Serum 25-OH-vitamin D levels (n=255) and PTH levels (n=243) were measured in 31 healthy common marmosets between the ages of 5 and 38 months. Of these, respectively 211 and 199 values were from animals younger than 21 months, and 44 were from animals 21 months or older. Common marmosets reach maturity at 21 months.

Results: Vitamin D levels showed a high variation (223.3 nmol/L ± 109.9) in the total population. Marmosets younger than 21 months had statistically significant ($p<0.0001$) lower vitamin D levels and a much wider range in values (205.2 nmol/L ± 102.7) compared to mature animals (330.9 nmol/L ± 77.76). A high variation in PTH levels (9.3 pmol/L ± 8.3) was also seen in the total population, but no statistical difference was observed between mature and immature animals.

Conclusions: The low vitamin D levels in immature marmosets are very remarkable. Vitamin D is necessary for skeletal growth and serum levels are generally higher in (healthy) children than in adults. None of the marmosets in our study population showed skeletal abnormalities, or any other illnesses.

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RESPIRATORY INFECTION ROUTES OF MERS-COV IN RABBITS

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Introduction: Middle East respiratory coronavirus (MERS-CoV) is causing respiratory disease in humans, often resulting in severe or even fatal pneumonia. Rabbits are susceptible for MERS-CoV after simultaneous intratracheal and intranasal inoculation with presence of virus in the nose and lungs. Therefore, rabbits are a useful animal model to study pathogenesis and preventive and therapeutic countermeasures for humans. However, virus tropism and course of disease in rabbits infected via solely the nose are unknown.

Materials and Methods: To compare different inoculation routes, 12 rabbits were divided in four groups and inoculated with MERS-CoV as follows: intratracheal with 3 ml and intranasal with 0.2 ml, intranasal with 1 ml or with 0.2 ml of MERS-CoV, or with 1 ml of PBS. Swabs were taken daily and at 4 days after inoculation the animals were euthanized, necropsied and sampled for histopathology, immunohistochemistry and virology.

Results: Rabbits inoculated via the intranasal route with 1 ml of virus demonstrated presence of infectious virus titers and high viral loads by PCR in nose swabs from 1 to 4 days after inoculation and in nasal turbinates and lungs at necropsy. Immunohistochemistry demonstrated virus antigen in nasal and pulmonary epithelial cells associated with inflammation.

Conclusions: This study demonstrates that rabbits can be infected via the intranasal route and have comparable virus tropism, virus load and inflammation as is seen in rabbits with additional intratracheal inoculation. Therefore, rabbits inoculated via the intranasal route can be used as animal model to study pathogenesis and intervention strategies for human disease.

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PERINATAL MORTALITY IN LOGGERHEAD TURTLE *(CARETTA CARETTA)* FROM A HEAD-STARTING PROGRAM

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Introduction: Head-starting is the practice of growing hatchlings in captivity to maximize survival during the first stages of development where mortality rates are higher.

Materials and Methods: Seventy eight neonates from a loggerhead turtle (*Caretta caretta*) died for various reasons in the development of a head-starting project during the first year of life

Results: Three epizootic outbreaks were observed. In the first one, the neonates had anorexia, lethargy, swelling and death (n = 14). Grossly, they showed a severe intestinal dilatation with large amounts of fecal liquid material. Histologically, they presented fibrinoecrotic and hemorrhagic enteritis with numerous bacterial colonies. Different bacteria were isolated, which is consistent with an intestinal dysbiosis. A second outbreak (n = 6) featured the massive infestation of the copepod *Balaenophilus manatorum*, which previously had been described as apparently harmless epibiont in sea turtles. Main lesions consisted of ulcerative and necrotizing dermatitis with intralesional parasites. The third outbreak caused the infection of all animals and the death of 36 individuals by *Amphiorchis spp*., an intravascular trematode of the *Spirorchiidae* family. The specimens showed progressive weakness, apathy and emaciation. Histologically, foreign body granulomas with numerous giant cells where observed in response to the massive presence of spirorchid eggs. Finally, in 22 individuals which were found dead (unrelated to the previously described outbreak), multiple granulomas, some of which had fungal hyphae, were found mostly in lungs.

Conclusions: Data provided in this study represents a new contribution to sea turtle pathology in head starting programs.

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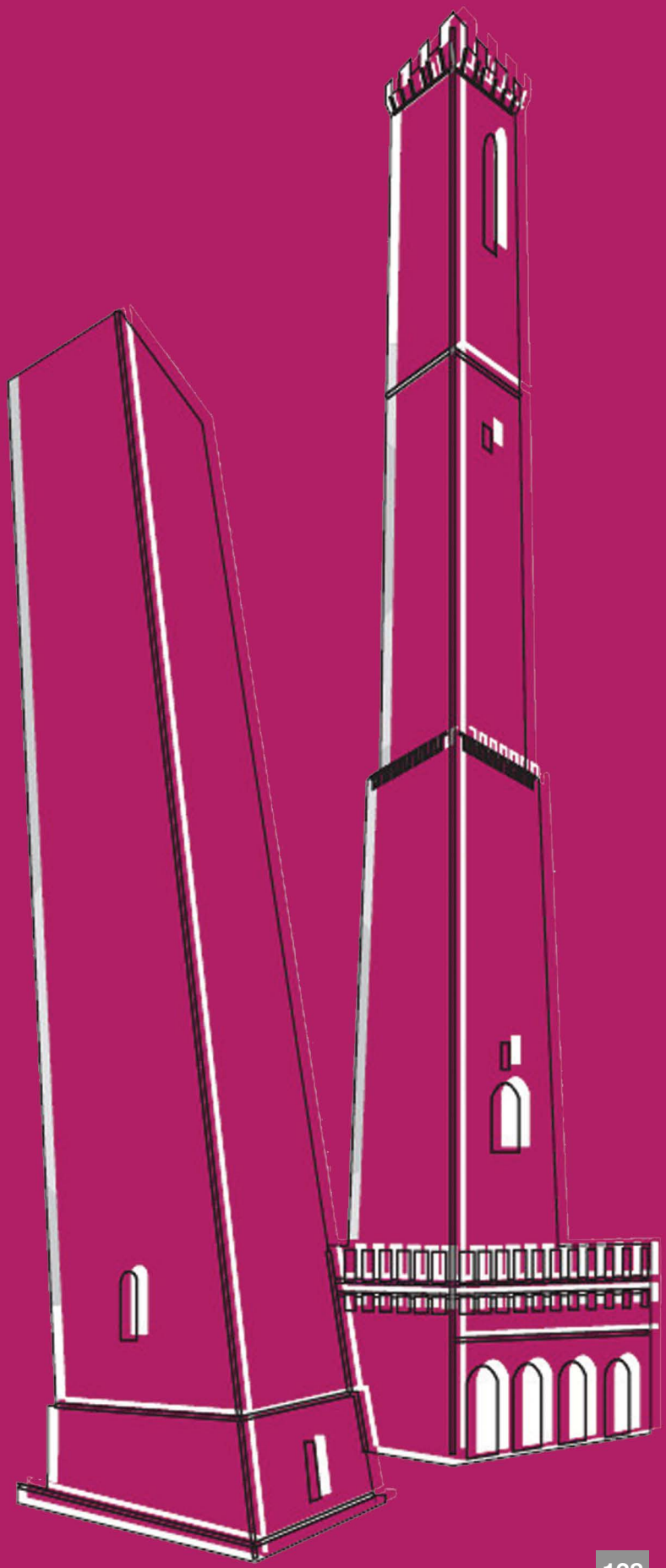

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APPLICATION OF MODIFIED SYDNEY SCALE IN THE DIAGNOSIS OF INFLAMMATION OF STOMACH IN DOGS

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Introduction: Gastritis in dogs is now a frequent problem with difficulties in diagnosis. The aim of this study was to apply the Modified Sydney Scale which is used in human medicine to describe mucosal inflammation in pylorus and body of stomach; including the infiltration of neutrophils, mononuclear cells, glandular atrophy and intestinal metaplasia.

Materials and methods: The sections of gastric mucosa obtained from 61 dogs were fixed in 7% formalin and embedded in paraffin blocks. The sections were stained with H&E and alcian blue.

Results: Within the pylorus infiltrations of granulocytes were observed: prominent in 3.3%, moderate in 6.6%, mild in 6.6% of cases. Infiltrations of mononuclear cells: prominent in 27.9%, moderate in 40.9%, mild in 27.9% of cases. Glandular atrophy: prominent in 1.6%, moderate in 16.4%, mild in 27.9% of cases. Intestinal metaplasia: moderate in 6.6%, mild in 1.6% of cases. Within the body of stomach infiltration of granulocytes: prominent in 4.4%, moderate in 4.9%, mild in 9.9% of cases. Infiltration of mononuclear cells: prominent in 26.2%, moderate in 24.6%, mild in 39.4% of cases. Glandular atrophy: prominent in 3.3%, moderate in 3.3%, mild in 18% of cases. Intestinal metaplasia: mild in 1.6% of cases.

Conclusions: This scale allows to specify the degree, type and intensity of inflammation in different parts of the gastric mucosa, which can help in choosing the best treatment option. In dogs mostly chronic inflammation with infiltration of mononuclear cells and the pyloric glands atrophy occurs. However, intestinal metaplasia is rare.

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MALAKOPLAKIA IN THE DIGESTIVE TRACT OF A DOG

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Introduction: Malakoplakia is a chronic inflammatory process which most often affects the urinary tract in immune-compromised human patients. Spontaneous animal cases of malakoplakia are rare having been reported in two pigs and a kitten.

Materials and Methods: A 12-year old male golden retriever with a one-week history of weight loss and inappetence was euthanized after clinical examination and transabdominal ultrasonography. Necropsy was performed and tissue samples for histopathological examination were stained with H&E, Periodic acid Schiff (PAS), toluidine blue, von Kossa, Prussian blue, Gram and Ziehl-Neelsen stains.

Results: Necropsy revealed thickened gastric wall (fundus and pylorus), white, firm nodules affecting greater omentum and gastrosplenic ligament and enlarged mesenteric lymph nodes. Histologically, severe fibrosis with multifocal round cell infiltrates mainly composed of histiocytes were present transmurally in the stomach, omentum and lymph node. Histiocytes were polygonal with abundant, granular eosinophilic cytoplasm that often contained round, basophilic cytoplasmic structures measuring from 2 to 20 µm (Michaelis-Gutmann bodies); some structures appeared homogenous while others had concentric, target-like appearance. Finely granular PAS-positive granules were present in the cytoplasm of histiocytes; strong PAS reaction was present in nearly all cytoplasmic bodies. Weak toluidine blue staining was present in cytoplasm of some histiocytes, while Prussian blue staining was very scant. Moderate to strong von Kossa staining was evident in areas of abundant histiocytic infiltrates. Gram and Ziehl-Neelsen stains were negative.

Conclusions: On the basis of histopathological features, this case was diagnosed as malakoplakia and to our knowledge, it is the first description of this condition in a dog.

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INFILTRATION AND SPATIAL DISTRIBUTION OF LYMPHOCYTE SUBPOPULATIONS AND CD117+ MAST CELLS IN FORMALIN-FIXED PARAFFIN EMBEDDED INTESTINE SAMPLES WITH CANINE INFLAMMATORY BOWEL DISEASE

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Introduction: Mediators released from mast cells may play a role in the development and maintenance of the inflammatory bowel disease in dogs. The activation of the transmembrane receptor KIT (CD117) is critical for the function of the mast cells and the release of its mediators. This research aims to quantify and evaluate changes in lymphocytes and mast cells distribution in intestinal samples of dogs with inflammatory bowel disease.

Materials and Methods: Lymphocyte immuno phenotyping and mast cell kit receptor expression in paraffin sections of formalin-fixed samples of small intestine with inflammatory bowel disease were analyzed by immunohistochemistry and computer assisted morphometric analysis was performed with an FSX Olympus microscope, and analyzed with the software Image ProPlus Tm.

Results: CD3+ lymphocytes and CD117+ mast cells were detected in significantly ($p<0.05$) higher numbers in the *lamina propia* of the intestinal tissue from dogs with an inflammatory bowel disease diagnoses when compared with samples from healthy dogs.

Conclusions: A higher number of infiltrative CD3+ lymphocytes in the *lamina propia* of the intestinal wall correlates with an increase in mast cells suggesting an altered Th1 immune response in dogs with inflammatory bowel disease. Also this investigation shows that the immuno histochemical demonstration of an increased CD117+ mast cell population could be a potential marker for canine inflammatory bowel disease.

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CONGENITAL ENTERIC ENDODERMAL CYST IN A PUPPY

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Introduction: Omental and mesenteric cysts are extremely rare findings in puppies. They can be classified by the histological observation of their walls into cysts of lymphatic, mesothelial, enteric or urogenital origin, among others. Here we report a case of an enteric endodermal cyst in a Labrador Retriever puppy.

Materials and Methods: An 8 month old Labrador Retriever puppy was presented for examination to a veterinary surgeon due to history of vomiting and anorexia during three days. Clinical general examination and abdominal X-ray and ultrasonography studies were performed. A mesenteric mass was extracted by midline laparotomy and was submitted for pathological examination. The mass was assessed grossly and was processed routinely by H&E, Masson´s trichrome and IHC for cytokeratin AE1/AE3.

Results: X-ray studies showed a round mass near the duodenum with no signs of obstruction. By ultrasonography, a multilayered wall surrounding an hypo-echoic cavity was seen. During the surgery the clinician observed no association between the mass and the intestinal wall. Grossly, the mass consisted of a unilocular 6 cm in diameter cyst. Histologically, the inner stratum of the cyst wall consisted of a layer of cuboidal to columnar, multifocally sloughed or degenerated, cytokeratin-positive epithelial cells. There was submucosa and wide double muscular layers with diffuse mild fibrosis.

Conclusions: We classified this structure as an endodermal cyst of enteric origin. It may be originated from an embryonic intestinal diverticulum that may have developed stenosis. Further reports of these rare malformations are needed in order to understand their origin and pathogenesis in dogs.

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EVALUATION OF *HELICOBACTER* SPP. IN THE INTESTINAL TRACT OF COMMON MARMOSETS (CALLITHRIX JACCHUS) WITH ENTERIC INFLAMMATORY DISEASE

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Introduction: Marmosets (*Callithrix jaachus*) in captivity are highly susceptible to develop enteric inflammatory disease. The primary aetiology of intestinal inflammation remains unclear. Hypotheses include allergic reactions against nutritional ingredients and bacterial infections, in particular Helicobacter spp. The aim of this study was the characterization of inflammatory lesions and their distribution in the intestinal tract of the common marmoset, and to investigate a possible correlation between Helicobacter spp. infection and these inflammatory lesions.

Materials and Methods: The small and large intestine from twenty-three common marmosets (mean age 6 ± 2 years) were microscopically (H&E) evaluated, and screened for detection of *Helicobacter* spp. antigen (immunohistochemistry) and DNA (*Helicobacter* spp PCR assay).

Results: Histologically, 21 out of 23 marmosets had inflammatory changes throughout the intestinal tract, with higher incidence and severity in the small (16/23 marmosets, from minimal to severe) compared to the large intestine (12/23, from minimal to mild). These changes were consistent with chronic, diffuse, lymphocytic and plasmacytic enteritis or enterotyphlocolitis. Immunohistochemical and molecular analysis revealed presence of *Helicobacter* spp. mainly in the large intestine.

Conclusions: In conclusion, there was no correlation between inflammatory lesions and presence of *Helicobacter* spp., indicating that *Helicobacter* spp. infection can be ruled out as the aetiology of enteric inflammatory disease in marmoset.

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ASPERGILLUS FUMIGATUS INFECTION IN AN OSTRICH WITH NECK INFLATED BY RESPIRATORY PROBLEMS

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Introduction: Aspergillosis is opportunistic infection in animals and humans caused by the genus *Aspergillus*. *Aspergillus* species are distributed widely in the environment such as soil, air and decaying vegetation. Fungi are identified mainly on the basis of the morphology of hyphae. This study describes a case of sudden death in an ostrich with an enlarged neck.

Materials and Methods: A 1-year-old ostrich was submitted for diagnosis. After necropsy, most organs and tissues were fixed in 10% buffered formalin, embedded in paraffin, and sectioned at 4 μm. The sections were then stained with hematoxylin and eosin for histopathology. For fungal examination, the air sacs and lungs were cultured aerobically on Sabouraud dextrose agar at 25 degrees. The fungus was suspended in lactophenol and examined microscopically by slide culture.

Results: Grossly, air sacs were thickened. Yellow to white round or coalescent materials were scattered on air sacs. However, the neck air sac was normal. Yellowish to white foci were multifocally present in the lungs. Histopathologically, multinucleated giant cells, lymphocytes and macrophages were infiltrating the air sac, and lots of hyphae were seen in the air sac and on its surface. Granulomatous inflammation with intralesional hyphae were multifocally observed in the lungs. Hyphae were positively r PAS stained. Conidiophore and conidia, characteristics of *Aspergillus fumigatus*, were identified by direct stamp and slide culture.

Conclusions: Based on the results, this case was diagnosed as *Aspergillus fumigatus* infection in an ostrich. The swollen neck was consider to be induced by air flow in air sacs.

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PATHOLOGY OF A H5 HIGHLY PATHOGENIC AVIAN INFLUENZA OUTBREAK FIELD CASES IN THE SOUTH-WEST OF FRANCE

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Introduction: Five field cases of H5 Highly Pathogenic Avian Influenza (HPAI) infections in Guinea fowl and chicken were investigated during outbreaks in the south-west of France from November 2015 to January 2016, using histopathology and viral titration.

Materials and Methods: On each case, up to 5 dead or moribund birds were selected and necropsied. On each bird, gross lesions were recorded and tissues were sampled for histopathology and virology. Formalin-fixed samples were routinely processed and analysed, and immunohistochemistry against Influenza A virus nucleoprotein (NP) was performed. Viral loads in tissues were determined by quantitative real-time RT-PCR on M gene.

Results: The overall mortality remained low to moderate and clinical signs were observed in a very limited proportion of birds, including oedema of wattles and cutaneous suffusions. On necropsy, lesions of subcutaneous and pulmonary oedema with congestion, and splenomegaly, were inconsistently observed. The main microscopic lesions consisted in systemic cutaneous and visceral tissue hyperaemia, marked acute interstitial pneumonia with capillary thrombosis and heterophilic leukostasis, mild lymphocytic portal hepatitis, and non-specific reactive splenitis. Lesions were more severe in Guinea fowls than in chickens. Immunohistochemistry analysis against influenza NP revealed an intense nuclear labelling, mainly of circulating leukocytes and endothelial cells. In the same way, quantitative PCR confirmed very high viral loads in most tissues.

Conclusions: To our knowledge, this is the first pathological investigation on the recent outbreaks of field H5 HPAI in France.

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HISTOLOGICAL, MORPHOMETRIC AND HISTOCHEMICAL FINDINGS IN BROILER CHICKENS FED DIETS WITH INSECT MEAL INCLUSION

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Introduction: Insects are considered a novel and suitable protein source for poultry feeding. Dietary modifications have been reported to affect intestinal morphology and mucin composition in broilers, but no studies related to insect meal utilization are currently available. The present study aimed to investigate histological findings, gut morphology and mucin composition in broilers fed with insects.

Materials and Methods: 40 male broiler chickens were distributed over 4 dietary treatments (control feed and 5%, 10% and 15% *Tenebrio molitor* inclusion) and slaughtered after 53 days. Spleen, thymus, bursa of Fabricius, liver, glandular stomach, intestine, heart and kidney were submitted to histological examination. Intestinal morphology was assessed through morphometric measurements of villus height, crypt depth and villus height/crypt depth ratio on duodenum, jejunum and ileum. Small intestine and caecum were also stained with PAS, Alcian Blue pH 2.5 and Alcian HID to discriminate among neutral, sialylated, and sulfated acidic mucins.

Results: Histological findings and intestinal morphology and mucin composition were not significantly influenced by dietary *Tenebrio molitor* inclusion. Lymphoid system activation and higher duodenal and jejunal morphometric indexes compared with ileum were observed in both control and insects feed. Neutral and acidic mucins stained similarly in all the treatments. Mucin staining was also more intense in the crypt base and midsection than tip and distally increased along the duodenal-ileal axis.

Conclusions: Dietary insect meal inclusion does not affect histological findings and gut morphology and mucin composition of the broilers, thus suggesting no negative influence on animal health and intestinal development.

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DEEP PECTORAL MYOPATHY IN TURKEY BROILERS

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Introduction: The development of modern methods for meat poultry breeding focused on achievement of high gains in bird's body weight and muscles increases the risk of myopathies and accompanying meat quality deviations, which can be a cause of substantial economic losses. The aim of the study was an analysis of the macroscopic and microscopic lesions of pectoral muscles of turkey broilers confiscated by veterinary service during post-mortem inspection due to their unusual appearance.

Materials and Methods: The analyses were performed on turkey breast fillets exhibiting a changed colour and texture of muscles, which were investigated macroscopically and sectioned for histopathological examination. After fixation in 4% buffered formalin, microscopic slides were made from the affected tissue samples using the paraffin method and stained with hematoxylin and eosin and histochemical methods.

Results: In the deep pectoral muscles, numerous small extravasations sites and white-grey-green fibrous foci were detected. The microscopic examination revealed multifocal confluent degenerative lesions turning into necrotic areas with inflammatory reaction accompanied by proliferation of connective tissue. The macro- and microscopic changes indicated necrosis of the pectoral minor muscle (*necrosis musculi pectoral minoris*).

Conclusions: The nature and sequence of the pathomorphological changes in combination with the features of pectoral muscle anatomy in turkey broilers suggest involvement of local circulatory disturbances in the aetiology of the diseases.

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VESSEL AND MYOFIBER DENSITY WITHIN FOCAL WOODEN BREAST MYOPATHY

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Introduction: Wooden breast myopathy (WB) of broiler chickens denotes a hardened consistency and paleness of the Pectoralis major muscle. The microscopic hallmarks consist of a polyphasic myodegeneration and perivascular lymphocyte aggregations. Hypoxia and relative insufficiency of blood supply may contribute to the pathomechanism of WB.

Materials and Methods: 14 experimental broiler chickens of a rapid-growth hybrid with a focally restricted WB lesion in their Pectoralis major muscle were euthanized at 18-38 days of age. The macroscopically unaffected (non-lesion) and the macroscopically affected (lesion) sites of each bird were sampled, processed into histological sections and stained with H&E and PAS-diastase. Muscle fibers and small-caliber vessels were counted on eight cross-sectional microscopic fields (MF) per site. Additionally, the lesion morphology was evaluated.

Results: The macroscopic lesion site expressed a slightly lower number of small-caliber blood vessels per MF (24.2±4.69; Mean±SD) than the non-lesion site (26.0±5.92; P = .046; d = 0.59). The number of myofibers per MF at the lesion site (53±23.50) showed no significant difference from the non-lesion site (48±8.56; P = .073). However, the individually normalized vessel-to-myofiber ratio was 0.6±0.10 at the lesion site and 0.5±0.15 at the non-lesion site (P = .051). The lesion site exhibited more severe and variable morphologic changes than the non-lesion site.

Conclusions: The blood vessel density is altered during the WB lesion development and neovascularization contributes to the vessel number. The high variation of myofiber number is partially explained by the different stages of polyphasic myodegeneration, especially in the lesion site of the muscle.

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41 CONSECUTIVE CHALLENGES WITH GENETICALY MODIFIED H7N7 LPAIV AND H7N7 HPAIV IN LAYER HENS

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Introduction: Avian Influenza is a notifiable and zoonotic disease and a severe threat to poultry industry. In 2008 highly pathogenic avian influenza virus (HPAIV) was detected in Oxfordshire, England, and although only HPAIV was isolated from the premises, viral RNA containing cleavage site motifs consistent with low pathogenicity (LPAIV) were found. These viral sequences were used to generate putative LPAI precursor viruses by reverse genetics (RG) from the wild type HPAIV. This study describes their infection in layer hens.

Materials and Methods: Three groups of layer hens were challenged with two RG precursor viruses derived from H7N7 A/chicken/England/08 HPAIV (G1 and G2) and a related contemporary LPAIV H7N7 A/mallard/Sweden/08 (G3). Two birds per group were sampled at 2 and 4 days post infection (dpi). At 14 dpi, all birds and 10 additional naïve hens (G4) were challenged with HPAIV H7N7 A/chicken/England/08. Post mortems were conducted at 2 dpi, on casualties and survivors at 14 dpi, followed by histopathology and viral detection by immunohistochemistry.

Results: Following LPAIV infection, no histopathology or immunolabelling were observed. Following HPAIV challenge with A/chicken/England/08, G1, G2 and G4 displayed similar systemic and widespread viral antigen in endothelial and parenchymal cells, but no immunolabelling was observed in G3. G1 and G2 presented lower mortality, but casualties within G2, G3 and G4 displayed similar histopathology and systemic viral distribution.

Conclusions: Previous RGs LPAIVs exposure resulted in decreased mortality after HPAIV challenge, but there were no major differences in pathology or viral antigen distribution in birds succumbing to disease.

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DIFFERENT EFFICACY OF INACTIVATED PANDEMIC 2009 H1N1 INFLUENZA A VIRUS VACCINES AFTER HOMOLOGOUS INFECTION IN FERRETS

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Introduction: Since its emergence, the (H1N1)pdm09 influenza A virus (IAV) has been established worldwide and replaced the previous seasonal H1N1 viruses, becoming the most isolated strain in recent years. The extent to which (H1N1)pdm09 influenza vaccines prevent viral infection and disease remains poorly understood. Hence, we evaluated the effectiveness of two (H1N1)pdm09 influenza human vaccines in ferrets.

Materials and Methods: Twelve influenza naïve ferrets were divided in two groups (V1 and V2) and immunised intramuscularly with two different A/California/07/2009 derived inactivated vaccines. Six weeks later, all animals were intranasally challenged with $10^{6.5}$ $TCID_{50}$ of the A/England/195/09 (H1N1)pdm09 isolate. A non-vaccinated (NV) challenged group of 6 animals was used as an infection control. Clinical signs, lung histopathology, viral quantification and antibody responses were evaluated.

Results: The V1 group showed reduced viral loads, milder clinical signs and histopathological scores. In contrast, V2 vaccinated animals exhibited higher clinical scores and enhanced viral shedding than the NV and V1 groups. V2 vaccinated animals presented more severe histopathological lesions in correlation with higher amounts of IAV IHC positive cells in the lungs.

Conclusions: There were important qualitative differences in the performance of both inactivated vaccines in relation to protection against challenge with a novel virus in a naive animal (ferret) model of human disease. V1 vaccine limited and controlled viral shedding and reduced lower respiratory tract infection, In contrast, V2 vaccine did not protect against infection and appeared to enhance early viral shedding and delay lower respiratory viral infection, resulting in more severe histopathological lesions.

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COMPARATIVE INFECTION OF NEWCASTLE DISEASE VIRUS IN CHICKENS, PHEASANTS AND PARTRIDGES

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Introduction: Newcastle disease is a highly contagious avian disease caused by Newcastle disease virus (NDV), the virulent form of avian paramyxovirus type 1. This study evaluates the pathogenesis of a divergent NDV lineage 5a-like serotype in chickens, pheasants and partridges which is currently circulating in Eastern Europe.

Materials and Methods: Juvenile chickens, pheasant and partridges were infected experimentally by the intraocular and intranasal route with 10^6 EID_{50} of APMV-1/chicken/BG/112/13. Two chickens were killed daily between 1 and 4 days post infection (dpi) whereas pheasants and partridges were killed at 2, 4, 7 and 9 dpi. Post mortem examination, histopathology and viral detection by immunohistochemistry were carried out.

Results: Clinical signs ranging from non-specific depression to severe neurological presentations nervous were observed in all three species. Mortality reached 100% in chickens and pheasants, compared to60% mortality in partridges. Pathological changes were most severe in pheasants where the virus was markedly lymphotropic, with abundant viral antigen and severe necrosis in lymphoid tissue, including the epithelium of the respiratory and digestive mucosa and neurons were also infected. Similar but milder findings were observed in chickens. In partridges, there was minimal replication in the lymphoid tissue (mainly caecal tonsil) with limited tissue distribution. Viral replication and marked inflammatory changes in the encephalon were characteristic of the end stage of this disease.

Conclusions: An emergent NDV strain that is currently circulating in Europe was able to produce severe disease in the three species studied, with marked lymphotropism in pheasant and chickens and neurotropism in partridges.

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STIMULATION OF LOCAL IMMUNE RESPONSES IN THE CAECUM AFTER APPLICATION OF A PROBIOTIC STRAIN IN CHICKENS

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Introduction: *Campylobacter jejuni* is zoonotic foodborne pathogen causing acute gastroenteritis in humans. Chickens are natural host for *Campylobacter* species. Secretory IgA antibodies in the intestinal tract form the first line of antigen-specific immune defence. IgM is the major class of immunoglobulin expressed on the surface of chicken B lymphocytes. CD3 molecules are important during the assembly of TCR complex. We quantified the numbers of mucosal IgA+, IgM+ and CD3+ cells in the chicken's caecum after administration of probiotic strain (*E. faecium* AL41) and *C. jejuni* CCM6191 (provided by Dr. Lauková, IAP, SAS, Slovakia).

Materials and Methods: Day-old chickens (n = 40) were divided into four groups: control (C), *E. faecium* AL41 (EFAL41), *C. jejuni* CCM6191 (CJ), and combined *E. faecium* AL41 + *C. jejuni* CCM6191 (EFAL41+CJ). Unlabelled primary mouse anti-chicken monoclonal antibodies IgA, IgM and CD3 were used.

Results: IgA+ cells showed the highest density in the EFAL41 group when compared to control, CJ and EFAL41+CJ group ($P < 0.001$) at 7 days p. i. The highest density of IgM+ cells was detected in EFAL41 group compared to control ($P < 0.001$) at 7 days p. i. The CD3+ cells showed the highest density in EFAL41+CJ group compared to control, EFAL41 and CJ group ($P < 0.001$) at 7 dpi.

Conclusions: Application of probiotic strain EFAL41 resulted in stimulation of local immunocompetent cells and was mostly present at 7 days p. i. Selected probiotic strains could influence the local immune response in caecum and thus reduces risk of campylobacteriosis.

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STIMULATION OF PROINFLAMMATORY CYTOKINES IN CHICKENS AFTER PROBIOTIC TREATMENT AND CHALLENGE WITH *C. JEJUNI*

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Introduction: *Campylobacter jejuni* is a major food-borne bacterial pathogen. Chickens are considered to be the main source of human *Campylobacter* infection. *Enterococci*, a group of lactic acid bacteria, are ubiquitous microbiota, which constitute a large proportion of autochthonous microflora found in the gastrointestinal tract of human and animals. The aim of this study was to investigate the effect of *Enterococcus faecium* AL41 on transcription of genes encoding proinflammatory cytokine (IL-15, IL-18, LITAF) in caecum of chicks after challenge with *Campylobacter jejuni* CCM6191**.**

Materials and Methods: One-day-old chicks (n= 60) were divided into 4 groups: control (C), EFAL41 (10^9 CFU/0.2 ml; from day 1 to 7), CJ (10^8 CFU/0.2 ml per os on day 4) and EFAL41+CJ. Samples of caecum were homogenized ant total RNA was isolated. Primers for cytokines were used and quantified by real time PCR. Amplification and detection of specific products was performed using the CFX 96RT system (Bio-Rad, USA) with predefined programme.

Results: Relative mRNA expression from the gene encoding cytokines were upregulated in the EFAL41+CJ group compared with other groups ($P<0.05$, $P<0.01$, $P<0.001$) at all sampling (1, 2 and 3 days post infection; dpi). Similarly, mRNA expression of IL-18 was upregulated in EFAL41+CJ group compared with other groups ($P<0.001$) at all samplings and LITAF expression was highest in the same group at 2 and 3 dpi.

Conclusions: Upregulation of proinflammatory cytokines suggest immunostimulatory effect of *E. faecium* on caecum of chicks infected by *C. jejuni*.

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MORPHOLOGICAL CHANGES OF OSTEOARTHRITIS IN FELINE STIFLE JOINTS AND ASSOCIATIONS TO INTRA-ARTICULAR MINERALIZATION

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Introduction: Osteoarthritis (OA) is common in cats. However, there are no detailed histological descriptions of feline stifle OA. Intra-articular mineralization (IAM) is commonly detected in feline stifle radiographs, but the association between small IAMs and feline OA is unclear. The purpose of this study was to describe OA lesions in feline stifle joints and investigate associations between articular cartilage lesions, synovitis and IAMs.

Materials and Methods: The right stifle joints from 29 cats (age 1-23 years, median 9) were examined for IAM using computed tomography and radiography, followed by macro- and microscopic evaluation of joint tissues. Articular cartilage lesions and synovitis were histologically graded. Cartilage lesions were summarized into global joint scores (GJS, n=28). Associations between cartilage lesions, synovitis and IAMs were determined.

Results: The most frequent location of cartilage lesions was the tibia (27/29 joints), followed by the patella (14/28 joints). The majority of cartilage lesions were low-grade. Femoral cartilage lesions (2/29 joints) were only seen in joints with severe tibial cartilage lesions. Synovitis was present in 13/29 joints and always low-grade. IAMs were detected in 13 joints, 11 were classified as small. Neither GJS nor synovitis scores were associated with IAMs, however increased GJS was associated with synovitis (P=0.001).

Conclusions: Osteoarthritis lesions in the articular cartilage were common and associated with synovitis. Tibial cartilage may represent the most common site for detection of early morphological cartilage changes of feline stifle OA. Lack of association between GJS and IAMs suggests that small IAMs are incidental findings in OA-affected joints.

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OSTEOPETROSIS-LIKE SYNDROME IN AN ENGLISH COCKER SPANIEL

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Introduction: A 3-year-old male-neutered English Cocker Spaniel with chronic kidney disease (CKD), non-regenerative anaemia and metastatic calcification underwent post mortem examination. Ante-mortem radiography showed diffuse osteosclerosis and hyperostosis. Congenital or acquired osteopetrosis-like syndrome was suspected.

Materials and Methods: Gross findings were reported and a complete set of tissues, including bone (skull and femur), was histologically examined. PCR and immunohistochemistry for canine distemper (CDV) were performed on lung and brain.

Results: Radiographic and gross examination showed hyperostosis predominantly affecting the skull, mandible and long bones. Histopathology of the skull and femur showed increased amount of compact bone. Trabeculae of the femoral cancellous bone were over-represented with central cores of mineralised cartilage (primary spongiosa) narrowing the marrow spaces. Osteoclasts were diffusely reduced in number, principally in the skull. The few osteoclasts observed appeared separated from the bone surfaces and not associated with obvious Howship's lacunae. Histopathology additionally showed end stage CKD and metastatic calcification of the myocardium, lung, gastric mucosa and plantar/palmar dermis. PCR and immunohistochemistry for CDV was negative.

Conclusion: Radiography, gross and histopathological examination of bone supports a diagnosis of congenital osteopetrosis-like syndrome, despite the subject's age. This dog also suffered from severe CKD, which was the likely cause of the metastatic calcification. As the consequences of CKD on bone metabolism are associated with bone resorption by osteoclasts (fibrous osteodistrophy) rather than osteosclerosis, it is possible that two different processes (CKD and osteopetrosis-like syndrome) were present in this subject, reinforcing the hypothesis of a congenital impaired osteoclast activity.

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OSTEOARTICULAR BOVINE TUBERCULOSIS IN PIGS (POTT DISEASE)

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Introduction: Pott disease, also known as tuberculous spondylitis, is a form of extrapulmonary human tuberculosis observed in the spinal vertebrae. The aim of this study is to describe some forms of bone tuberculosis in pigs slaughtered in the Nebrodi park (Sicily).

Materials and Methods: During a slaughterhouse survey one hundred fifty-five carcasses of pigs of 8-24 months old were examined. Necrotic lesions observed in the skeletons were considered as suspected tuberculous lesions and submitted for X ray examination, histopathology and bacteriological analysis.

Results: Macroscopically, 7 carcasses showed diffuse areas of caseous necrosis mainly involving the axial skeleton and the ribs. The most affected were the spinous processes and vertebral bodies with compression of the spinal cord in two cases. The lesions appeared variable in shape and dimension and were identified in the cervical, thoracic, lumbar e sacral tract of the vertebral column. The X rays showed multifocal areas of osteolysis also involving the appendicular skeleton. Histologically the tubercle contained a central core of necrotic tissue, surrounded by epithelioid cells and rare Langhans giant cells. More peripherally lymphocytes, macrophages and varying degrees of fibroplasia and encapsulation were also observed. All isolates were identified as *Mycobacterium bovis*.

Conclusions: To the best of author's knowledge there are no reported cases of Pott disease in pigs.

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CORRELATIVE ANALYSIS OF SYNOVITIS AND DEGENERATIVE CRUCIATE LIGAMENT ALTERATIONS IN DOGS WITH NON-RUPTURED CRANIAL CRUCIATE LIGAMENTS

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Introduction: Lymphoplasmacytic synovitis has been suggested to be an important factor initiating and/or promoting cranial cruciate ligament (crCL) degeneration leading to spontaneous rupture of the ligament. So far, there is only limited information about synovial inflammatory changes in dogs with macroscopically intact crCLs. The aim of this study was to evaluate the histological findings in synovial samples and crCLS from dogs with non-ruptured crCLs.

Materials and Methods: Synovial samples and crCLs from 72 dogs of different age, body weight and breed euthanased for reasons unrelated to the joints were examined histologically. The severity of degenerative crCL alterations and of synovitis was evaluated by histology and synovial inflammatory cells were labelled immunohistochemically.

Results: In 25/72 dogs, both crCL alterations and synovitis were present. Statistically, a positive correlation between severity of synovitis and severity of crCL lesions was found. In 15/72 dogs, only crCL alterations were present and 5/72 dogs had synovitis but normal crCLs. Tissue samples of 27 dogs were histologically unremarkable. In the inflamed synovium, beside T and B lymphocytes and plasma cells, numerous CD163+ and S100A8/S100A9+ macrophages were detected.

Conclusions: The results suggest that synovitis of the canine stifle joint synovium apparently is not the primary event, but possibly an early feature in the development of degenerative crCL lesions. Furthermore, the results indicate that macrophages may be involved in the pathogenesis of crCL alterations. The data support results of other investigations, in which advanced age and higher body weight were identified as risks factors for canine cruciate ligament disease.

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50 CANINE DILATED CARDIOMYOPATHY (DCM): FURTHER INSIGHTS INTO THE ASSOCIATED PATHOMECHANISMS

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Introduction: Dilated cardiomyopathy (DCM) is one of the most common cardiac diseases in dogs. DCM is associated with myocardial remodeling, and myocardial inflammation might be involved in the disease process. In humans, recent literature focused on the role of stem cells (SC) in cardiac repair and regeneration, while in dogs the pathomechanisms underlying DCM are not yet fully understood. The aim of the present study was to assess in more detail the remodelling processes in canine DCM.

Materials and Methods: Histological specimens from the hearts of 17 dogs with DCM were evaluated by histology and immunohistology to assess any histological changes and identify the cell types present as well as any functional changes in cardiomyocytes.

Results: A number of histopathological changes of variable intensity was observed, ranging from interstitial oedema and an increase in interstitial cells, over neovascularisation to interstitial fibrosis and cardiomyocyte degeneration. The majority of interstitial cells appeared to be of macrophage origin and frequently expressed ICAM, TGF-β and VEGF, markers that were additionally identified in variable numbers of cardiomyocytes. Numerous α-SMA-positive myofibroblasts were present and occasional interstitial cells expressed the SC marker c-kit as well as troponin T. Furthermore, neovascularisation was a prominent feature.

Conclusions: The results of the present study indicate that several processes are involved in the pathogenesis of DCM, one being myocardial inflammation, others include myofibroblast proliferation and differentiation, fibrosis and neovascularisation. Few SC were identified; however, whether SC play a role in the pathogenesis of canine DCM requires further investigations with appropriate markers.

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51 RUPTURED AORTIC SINUS OF VALSALVA ANEURYSM ASSOCIATED WITH AORTIC CYSTIC MEDIAL DEGENERATION AND AORTO-CARDIAC FISTULA IN A HORSE

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Introduction: Ruptured aortic sinus of Valsalva aneurysm associated with a formation of aorto-cardiac fistula is a rare condition in horses. Rupture of the aortic root or aneurysm of the right aortic sinus can lead to an aorto-cardiac fistula, permitting communication between the aorta and cardiac chambers.

Materials and Methods: An 18-year-old Paint mare underwent clinical examination with electrocardiographic and echocardiographic examination due to depression, exercise intolerance, anorexia, tachycardia, fever and tachypnea. Because of an inadequate response to cardiological treatment, euthanasia was elected two months after the initial presentation. Heart gross post-mortem and histopathological examination (including H&E and PAS staining) was performed.

Results: Trans-thoracic echocardiography revealed an aneurysm of the right sinus of Valsalva protruding into the right atrium. Ruptured aneurysm and associated blood flow through and aorto-cardiac fistula was confirmed using color flow Doppler.

Post-mortem examination confirmed the communication between the right sinus of Valsalva and right atrium. The histopathological examination revealed fragmentation and hypoplasia of the elastic fibers in the aorta separated by PAS-positive interstitial homogenous material.

Conclusions: Although rarely noted, aorto-cardiac fistulas should be considered as a possible cause of heart failure symptoms in elderly horses.

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CARDIOVASCULAR HISTOPATHOLOGICAL CHANGES ASSOCIATED WITH CHRONICALLY IMPLANTED BLOOD PRESSURE TELEMETRY DEVICES IN MICE

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Introduction: Radiotelemetry offers continuous long-term blood pressure measurement in conscious, freely moving animals and thus is the gold standard for blood pressure analysis in rodents. This work presents the histopathological changes that can be induced by blood pressure catheters.

Materials and Methods: As part of the phenotypic assessment of the microsomal epoxide hydrolase (mEH) gene manipulation, twenty 3-month-old male C57BL/6 mice (wild type, knock-out and knock-in mice for *mEH*) were implanted with pressure sensing catheters into the left carotid artery and, 8 weeks post-surgery, underwent ultrasonographic examination, euthanasia and a thorough post mortem analysis with particular emphasis on the cardiovascular system.

Results: Ultrasound examination demonstrated obvious turbulence within the blood flow of the aortic arch in all animals, regardless of the genotype. Indeed, the tip of the catheter did occasionally strike the aortic wall as a result of pulsation during the cardiac cycle. In the majority of animals, the post mortem examination revealed focal proliferative lesions (increased wall thickness, cartilage metaplasia and intimal proliferation) of variable severity in the aortic arch; this was associated with partial luminal occlusion and thrombosis.

Conclusions: Our study provides strong evidence that implanted pressure sensing catheters frequently induce vascular changes. Without appropriate sham-operated controls and an alternative simultaneous way to measure blood pressure, it is impossible to assess the extent to which these lesions influence pressure measurements. However, our results suggest that telemetry blood pressure recordings in mice should be interpreted with caution and always in association with the results of the histological assessment.

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AORTO-CARDIAC FISTULA IN A WARMBLOOD STALLION

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Introduction: A 19-year-old Grand Prix dressage horse was admitted to the hospital with a history of colic, tachycardia, jugular pulsation, sweating and peripheral edema. A right-sided pansystolic and early mid-diastolic 3/6 murmur was noticed and electrocardiography revealed paroxysmal monomorphic ventricular tachycardia (140/min) and atrial fibrillation. After anti-arrhythmic treatment the general condition improved, but the horse suddenly died in the stable, 14 days after initial presentation.

Materials and Methods: Full post-mortem examination and histology were performed.

Results: Necropsy revealed cardiomegaly with multifocal subendocardial fibrosis in both the left and right sides of the heart. An aorto-cardiac fistula was present between the right aortic sinus of Valsalva and the right ventricle. The ruptured right sinus of Valsalva, but also the non-ruptured non-coronary sinus, showed an aneurysmal dilation. The aneurysmatic sinus wall consisted of dense fibrous tissue with loss of smooth muscle cells and elastin fibers.

Conclusions: In horses, but also in human patients, the right conary sinus is the most common site to dilate or to tear with rupture into the right atrium or ventricle. These coronary sinus aneurysms are considered to be congenital in humans with a predisposition for males. A congenital weakness in the wall of the sinus of Valsalva, as seen in humans, seems unlikely in our horse as a lack of continuity between aortic media and aortic annulus was not noticed. Further research is needed to elucidate the pathogenesis of this rare but often fatal disease in horses.

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PENICILLIUM GLABRUM GRANULOMA PRESENTING AS A HEART BASE MASS IN A CAT

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Introduction: A 12-year-old cat was presented with a 3-week cough and tachypnea. A CT-scan revealed the presence of a cranial mediastinal mass. The cat was euthanized because of a suspected heart base tumor.

Materials and Methods: At necropsy, FFPE tissue samples were collected and stained with H&E. DNA was extracted from the heart base mass, amplified with an ITS1-4 primer and the resulting DNA fragments were sequenced.

Results: A firm, white mass of 3 cm in diameter was present at the base of the heart and attached to the right atrium and the right cranial lung lobe. Randomly dispersed white nodules, varying between 3 mm and 3 cm, were spread throughout the lungs.

Histologically, the mass consisted of large areas of necrosis containing lightly basophilic, septate, 8 µm wide hyphae, surrounded by an eosinophilic granulomatous inflammation. At the periphery of the mass, several large bronchi with transmural necrosis and inflammation were seen. Both in these bronchi and in other lung sections, large numbers of embryonated nematode eggs and lesser numbers of larvae, morphologically consistent with *Aelurostrongylus abstrusus*, were present. DNA sequencing allowed identification of the fungus as *Penicillium glabrum*.

Conclusions: This is the first report of infection of an animal or human with *P. glabrum*, which is normally known as a food contaminant. The route of infection of *P. glabrum* remains elusive. However, the involvement of the lung in the granuloma and the extensive lungworm infestation suggests primary pulmonary damage due to lungworms after which the fungus found a portal of entry.

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CHANGES IN IRON STATUS ASSESSED IN PERIPHERAL BLOOD AND LIVERS OF PIGS WITH TACHYCARDIA-INDUCED CARDIOMYOPATHY

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Introduction: Changes in iron status occurring during the natural history of heart failure (HF) and related mechanisms remain unknown. We examined iron status using porcine model of tachycardia induced cardiomyopathy.

Materials and Methods: Homogenous male siblings of White Large breed swine (n=12) underwent a continuous right ventricular pacing (RV) at 170 bpm, 4 sham-operated subjects served as controls. All pigs underwent euthanasia at subsequent stages of the disease (mild, moderate and severe HF). We analyzed indices of iron status in peripheral blood, Fe^{3+} amount in liver sections (Prussian blue reaction), and hepatic expression of ubiquitous intracellular protein that stores iron and releases it in a controlled fashion - ferritin light (FTL) and heavy (FTH) chain (immunohistochemistry).

Results: Local passive congestion was seen in the liver section in pigs with mild HF, whereas in moderate and severe HF massive passive congestion with the areas of necrosis was observed. The progression of HF was accompanied by a gradual decrease of Fe^{3+} in hepatocytes (R=-0.921, p=0.00015). HF was inversely correlated with transferrin saturation (TSAT) (R=-0.5699, p= 0.01), a marker of biological iron availability, when TSAT was associated with hepatic Fe^{3+} content (R=0.833, p=0.005). Ferritin (both FTL and HTL) amounts in hepatocytes was decreased in severe HF.

Conclusions: The development of HF due to RV pacing is accompanied by decreased hepatic Fe^{3+} content linked with lower biological iron availability as well as reduced hepatic iron storing ability (in severe HF).

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ATRIOVENTRICULAR VALVE DYSPLASIA IN COMMON FOXES (*VULPES VULPES*) WITH CARDIORESPIRATORY SYNDROME

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Introduction: Cardiorespiratory syndrome (also called lung-heart syndrome) among common foxes was described for the first time in mid 1980s in Poland and Canada and has been reported as a cause of significant losses in fox farms. The etiology of the disease still remains elusive. Our previous study suggests the possible role of heart defects in the etiology of the disease.

Materials and Methods: The study was conducted on 50 foxes that died with symptoms of acute cardiorespiratory failure at fox farm in years 2012 and 2013. Special emphasis was put on the detailed post-mortem examination of the animals' hearts.

Results: The pathological examination of the hearts revealed a dilatation of the right atrioventricular opening and abnormal structure of the tricuspid valve in all cases. The septal cusp was shortened, thickened, and immobilized. The tendinous chords of that cusp were completely absent in 29 hearts (58%), or significantly shortened in another 21 cases (42%). Moreover, in 32% of animals an abnormal structure of the mitral valve was noted.

Conclusions: The presence of tricuspid valve dysplasia in all examined animals suggests its role in the etiopathogenesis of cardiorespiratory syndrome.

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CARDIAC *TRUNCUS ARTERIOSUS* IN AN EASTERN BLACK RHINOCEROS (*DICEROS BICORNIS MICHAELI*)

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Introduction: A one-month-old female Eastern black rhinoceros (*Diceros bicornis michaeli*) born in captivity at Chester Zoo (UK), was presented at 1 month old but below the average expected weight for an animal at birth. She was reported with sudden onset lethargy and laboured breathing. This rapidly progressed to cardiorespiratory arrest with no response to resuscitation attempts.

Materials and Methods: The carcase underwent post-mortem examination and a full set of tissues was submitted for histopathological examination.

Results: Post-mortem examination showed severe enlargement and rounding of the cardiac profile, moderate hydrothorax and severe pulmonary congestion. The heart presented with a single arterial trunk originating from the right ventricle and giving origin to the aorta. A large 5x4cm irregular defect of the interventricular septum was also observed. The base of this arterial trunk exhibited a single valve characterised by 3 irregular and thickened leaflets from which a narrow fibrous sheet was continuous with the mitral valve through the septal defect. Approximately 3cm distal from the valve, the left and right pulmonary arteries arose independently from either side of the trunk. Histological examination revealed marked myocardial degeneration and chronic pulmonary congestion with abundant alveolar haemosiderophages (heart failure cells).

Conclusions: To our knowledge this is the first report describing cardiac malformation in an Eastern black rhinoceros. The morphological features of this malformation closely resemble type III *truncus arteriosus* in humans, which is associated with an early embryological defect on the outflow tract division of the foetal heart.

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DIFFUSE CYSTIC DYSPLASIA OF THE KIDNEY IN NORWICH TERRIERS

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Introduction: In Norwich terriers a syndrome consisting of polycystic kidneys and hepatic fibrosis is one of the diseases responsible for the high neonatal mortality in this breed. The disease is hereditary and we have identified a mutation for this disorder within a known ciliopathy gene. The aim is to characterize the renal and hepatic lesions in this syndrome.

Materials and Methods: A complete necropsy was performed on three affected newborn Norwich puppies from three different litters. For histology HE, Masson trichrome and Hall´s stain were used. For immunohistochemical staining the following antibodies were used: polyclonal rabbit anti-CD31, von Willebrand factor, anti-GSTA1, anti-GSTT1, anti-aquaporin-1 (AQP-1) and anti-aquaporin-2, monoclonal mouse anti-α-SMA and anti-calbindin-D28K, and polyclonal sheep anti-human Tamm-Horsfall protein.

Results: The portal areas of the liver were expanded and contained numerous bile ducts surrounded by α-SMA positive mesenchyme. There was no bile stasis. In the kidneys there was marked cystic dilatation of the tubules both in the cortex and medulla with multifocal loose α-SMA positive mesenchyme. The glomeruli were poorly developed. The cystic tubules stained positively with anti-AQP-1 antibodies which together with their localization and morphology was compatible with straight part of proximal tubules and thin descending limbs of Henle's loop.

Conclusions: The liver lesions were typical for ductal plate malformation and consistent with congenital hepatic fibrosis. The renal lesions were different from all previously described polycystic kidney diseases in humans and animals. The renal disease in Norwich terriers appears to be unique and can be best described as diffuse cystic dysplasia.

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PECTUS EXCAVATUM IN 7 DOGS-CLINICAL REVIEW

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Introduction: Pectus excavatum is a very rare congenital malformation of the sternum and related costal cartilages. The etiology of this disorder remains unclear, although it is assumed that there is genetic component for a least some cases.

Materials and Methods: 7 dogs of different breeds, gender and age were examined at the Radiology Department of the Brno Faculty of Veterinary Medicine between 2010-2016. The physical examination showed a marked inward deformity of caudal half of the ventral thorax, and severe respiratory distress especially when the dog was placed in dorsal recumbency. Lateral and ventrodorsal radiographic views of the chest were obtained.

Results: Lateral and vetrodorsal views showed mild to moderate dorsal displacement of the caudal sternebrae, left cardiac displacement in three dogs and right cardiac displacement in four dogs.

Conclusions: The diagnosis of pectus excavatum is based on an anamnesis, clinical signs, clinical examination and diagnostic imaging of the chest. The most reliable imaging techniques in the diagnosis of this disorder is a radiological examination, which allows for an accurate assessment and final diagnosis.

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STRUCTURAL CONGENITAL DISORDERS IN RATS IN TOXICOLOGICAL STUDIES

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Introduction: Congenital disorders (birth defects) are changes that occur in the body before birth regardless of their cause. They can be related to ongoing processes in the organism (functional defects, i.e. lack of an enzyme) or to the anatomy or histology (structural defects).

Materials and Methods: A gross examination of over 2500 rats was conducted. Samples of internal organs were collected, fixed in 10% neutral buffered formalin, embedded in paraffin, stained with haematoxylin-eosin (H&E), iron hematoxylin-periodic acid-Schiff reagent (PAS), and iron hematoxylin-acid fuchsin-phosphomolybdic acid-methyl blue (Masson's trichrome), and examined under a light microscope.

Results: During an autopsy single cases of hydronephrosis, malocclusions, lack of the right lobe of the thyroid with compensatory hypertrophy of the left lobe of the thyroid, hermaphroditism, underdevelopment of the testicles and / or epididymitis, uterus didelphys, polydactylia, supernumerary kidney, and abnormal position of internal organs were observed. A histopathological evaluation performed in some cases confirmed the results of the gross examination.

Conclusions: The occurrence of structural congenital disorders in rats in acute toxicity studies does not seem to be of significance for the test results. However, in the case of subchronic and chronic tests, the presence of some of the observed changes can cause false results. This is particularly important in reproductive toxicity and teratogenicity tests in which more frequent and undiscovered congenital defects of the reproductive system causing reduced fertility can require repeating the experiment.

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61 WHAT IS YOUR DIAGNOSIS? FINE NEEDLE ASPIRATE OF A PANCREATIC MASS IN A CAT

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Introduction: A 10 year-old intact female cat was presented to the Veterinary Teaching Hospital of the University of Cordoba for routine ovaryhysterectomy. During surgery, several brownish to red, round to oval nodules, with an average diameter of 0.5 cms, were observed in both pancreatic lobes. A fine-needle aspiration of the lesions was performed and a direct smear was stained with modified May-Grumwald Giemsa..

Results: Resulting smear presented a marked erythrocytic contamination and elevated cellularity. Most cells corresponded to small-medium sized lymphoid cells (mostly mature small lymphocytes and occasional lymphoblasts without any atypical form), conspicuous eosinophils and neutrophils with fewer histiocytes and plasma cells. Nucleated red blood cells could also be seen. Occasionally those cells were intermixed with numerous endothelial-lined vascular structures. Rare pancreatic acinic cells with normal features were also seen in groups. The cytological exam was consistent with pancreatic splenosis, which was confirmed histologically in a resected nodule.

Conclusions: Splenosis is a rare finding in veterinary. In cases of splenosis, the heteropic splenic tissue is commonly found on serous membranes, but intraparenchymal hepatic and pancreatic splenosis have also been reported. Cytologically, this condition can be confused with lymphoma, pancreatic or neuroendocrine neoplasms and is described to be a cause of unnecessary surgeries in human medicine. Reports of cytology on human splenosis show similar descriptions to our case. This case outlines the importance of cytology in the evaluation of any pancreatic abnormality and, to our knowledge, is the first description of the cytological findings of splenosis in veterinary.

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USEFULNESS AND ACCURACY OF FINE NEEDLE ASPIRATION CYTOLOGY IN INVESTIGATIONS OF THYROID ENLARGEMENTS IN DOGS

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Introduction: thyroid tumours are common in dog. Among diagnostic tools, Fine Needle Aspiration Cytology (FNAC) is anecdotally considered as a sensitive test but in veterinary literature there is a paucity of reports concerning data about its sensitivity, specificity and diagnostic accuracy. The aim of this study is to determine the usefulness and diagnostic accuracy of cytology of canine thyroid swellings.

Materials and Methods: The records of 28 dogs that underwent FNAC performed at our laboratory during the period January 2005 to December 2013 were retrieved; data about cytological and histopathological diagnosis were extracted and compared. Sensitivity, specificity and accuracy of FNAC were analysed .

Results: The correlation of the cytological diagnosis with the histopathological diagnosis showed that the cytological diagnostic accuracy rate was 95%, with a sensitivity of 95% and a specificity of 100%.

Conclusions: the results of our study demonstrate that 75% of cytological samplings were valuable and diagnostic for thyroid enlargement. When adequate, cytological sampling is a specific, sensitive and accurate initial diagnostic test for the preoperative evaluation of thyroid nodules .

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VON BRUNN'S NESTS IN THE URETER OF TWO CATS

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Introduction: Von Brunn's nests and cysts are submucosal benign urothelial processes, related to irritative stimuli. They are common findings in the urinary bladder of human beings. Very few cases have been described in dogs and cats.

Materials and Methods: We report macroscopic, histopathological and, in one case, immunohistochemical features of these rare forms in the left ureters of two cats, after ureterectomy due to urinary obstruction.

Results: In both cases, macroscopical findings were coherent with an isolated, nodularand compact mass of 2.5 cm diameter. Microscopic examination showed considerable thickening of the muscular wall due to multifocal fibrosis, multifocal lacking of the lining epithelium in transverse sections of the ureteral lumen, and foci of urothelial islands in the lamina propria without any cytological atypia or mitosis and with occasional central cystic cavities. Multifocal areas of oedema, hyperemia/hemorrhage, angiogenesis and mild to moderate widespread lymphoplasmacytic infiltrates were also present. The cystic cavities contained macrophages full of lipofuscin, hemosiderin, erythrocytes and eosinophilic amorphous material. In one case, urothelial islands showed intense immunoreactivity for pan-cytokeratin (CK AE1/AE3). Inflammation was poor in the other case, where the urothelium appeared multifocally dilated with focal mucous metaplasia. In both cases, such findings and the anamnesis allowed us to hypothesize that the presence of epithelial islands was consistent with a hyperplastic condition, known as von Brunn's nests and cysts.

Conclusions: It is important to know the histological features of von Brunn nests, since it is not always easy to distinguish these islands from a well-differentiated urothelial carcinoma.

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HISTOLOGICAL FINDINGS IN ULTRASOUND GUIDED HEPATIC BIOPSIES IN DOGS

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Introduction: Ultrasonography by itself can't establish a reliable diagnosis for liver diseases and requires a histological examination of the affected tissue. The hepatic biopsy offers the most convenient way of examining the liver tissue. The use of ultrasound guidance diminishes the risks of the procedure by avoiding the bile ducts and the large vascular structures and by monitoring any complications that might occur post-biopsy.

Materials and Methods: Ultrasound guided biopsies were performed under local anesthesia or procedural sedation using Tru-Cut biopsy core needles. The number of specimens from each individual was determined by the quality of the tissue sample macroscopically evaluated by the pathologist, or by the multifocal ultrasonographic features of the lesions. Tissue samples obtained from eleven dogs of ages between 6 and 15 years were formalin-fixed, paraffin-embedded and H&E stained.

Results: The most encountered lesion was hepatic steatosis (n=8), often accompanied by chronic inflammation. In the case of a 15 years old poodle, an additional rubeanic acid staining confirmed the suspicion for copper-associated chronic hepatitis. The presence of fibrosis, an important feature in staging the liver disease, varied from fibrous portal expansion to cirrhosis (n=1). Other lesions discovered included a cholangiocarcinoma and a presumed hepatic lymphoma.

Conclusions: The liver biopsy remains a golden standard in the evaluation of canine hepatic diseases, the histological examination still being the most reliable method of diagnosis.

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MORPHOLOGICAL LESIONS IN FERRETS' SPLEEN – CASE REPORTS

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Introduction: Ferrets (*Mustela putorius furo*) are very popular pets, however knowledge of their physiology and pathology is still incomplete due to the relatively late domestication. That is why ferrets are difficult patients in veterinary clinical practice. Our observations show that splenomegaly is common diagnosed pathology. The paper presents diversity of its causes.

Materials and methods: The main materials of the study were tissue samples from spleen, which were obtained through splenectomy of 7 ferrets of different age (4-7 years) and sex (3 male, 4 female). Samples were fixed in 10% neutral buffered formalin, subsequently dehydrated, embedded in paraffin and stained with haematoxylin-eosin. Afterwards specimens were analyzed by light microscopy and evaluated.

Results and discussion: The analysis revealed that the most common cause of splenomegaly was neoplasia like lymphoma (3), including lymphoma zone marginale (1). Among other diagnosed reasons are: *Mycobacterium spp.* infections (1), hematoma (1) and lymphoid hyperplasia (2). Observations from clinical practice suggest that splenomegaly is a relatively common cause of sudden deterioration of animals physical condition. Its causes may be quite varied - from infectious agents to cancer . Our personal observations show that in some cases splenectomy is a procedure which gives positive therapeutic effects.

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CLINICOPATHOLOGICAL FEATURES OF CANINE ABDOMINAL MASSES: 80 CASES

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Introduction: epidemiological data on canine abdominal masses are fragmentary and focused on specific diagnoses and site. The aim of this study is to describe the epidemiology, distribution and diagnosis of canine abdominal masses.

Materials and Methods: surgically excised abdominal masses with largest diameter ≥ 3cm and available histological diagnosis were collected. Dog signalment, tumour size and site were recorded. Hematoxylin and eosin stained slide were reviewed and, when necessary, immunohistochemistry was performed. Lesions were classified as malignant (ML) and benign (BL), the latter including benign neoplasia and non-neoplastic lesions.

Results: 80 lesions were collected in dogs between 1 and 15 years of age (median 11). M/F ratio was 1,7. Forty-nine cases were splenic, 14 gastrointestinal (GI), 10 intra-abdominal (without connection with any organ), 4 genital and 3 hepatic. Fifty were ML and 30 were BL, 24 of which non-neoplastic and 6 benign neoplasia. Thirty-seven ML (74%) were mesenchymal (21 of which hemangiosarcomas), 7 leukocytic (4 histiocytic sarcomas and 3 lymphomas), 3 epithelial and 3 miscellanea. ML were fewer in the spleen (55%) than in GI-tract (86%) and abdomen (70%) ($p<0.05$). Difference in age or sex was not evidenced between ML and BL.

Conclusions: the majority of canine abdominal masses in this study are mesenchymal ML occurring in middle age to old male dogs with a preponderant involvement of spleen, GI-tract and abdomen. The spleen was the site with the lower percentage of ML. These data might be useful for planning the approach to canine abdominal masses in every-day practice.

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DIFFERENTIAL DIAGNOSIS OF NEOPLASTIC-LIKE LESIONS IN WILD ANIMALS

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Introduction: Neoplasms are frequently described in captive wild animals due to management improvements and longer life expectancy. In fact, it is possible that many of these neoplastic suspicions are lesions of other nature, requiring an accurate differential diagnosis.

Materials and Methods: Thirty biopsy samples from captive wild animals with neoplastic suspicions and housed in the Wildlife Screening Center (São Paulo, Brazil) were evaluated by histochemical and immunohistochemical techniques, as well as by transmission electron microscopy and by PCR when necessary, for etiological confirmation.

Results: Eleven biopsies (36.7%, 6 birds, 3 reptiles, 1 amphibian, 1 mammalian) indicated infectious ethiologies; 63.3% presented neoplasms. All lesions were proliferative and unresponsive to conventional treatments. Samples from 1 passerine, 1 strigid and 1 psittacid showed typical mycobacterial granulomas. Mycobacteriosis was also diagnosed after the amputation of a forelimb from a frog with nodular ulcer. A ramphastid presented a cutaneous granuloma in the tibial-tarsal joint compatible with cutaneous leishmaniasis, showing both amastigote and promastigote forms. A snake and a non-human primate also exhibited granulomatous infiltrates associated with septate hyphae stained with Grocott suggesting filamentous fungal infection. Periocular poxviral disease was found in a passerine exhibiting intracytoplasmic eosinophilic inclusions. An anatid presented a nasal pedunculated and ulcerated polype, with multiple sporangia of *Rhinospordium seeberi*. A chelonian with suspected liver adenocarcinoma presented hepatic lipidosis and fibrosis. Finally, a lizzard diagnosed with oviduct adenocarcinoma with pulmonary/renal metastasis, co-presented mycobacteriosis.

Conclusions: In wild animals, some suspected neoplastic lesions are in fact of infectious origin and precise diagnosis requires technical expertise.

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SEARCHING FOR NEW BIOMARKERS OF ESTROGEN TREATMENT IN BOVINE FAT AND MUSCLE

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Introduction: In order to identify potential biomarkers, effects of estrogen treatment on skeletal muscle and adipose tissue samples were investigated.

Materials and Methods: Twelve Friesian beef cattle were treated as follows: group R (n=6) Revalor-200 (trenbolone-acetate and estradiol) subcutaneous implant for 89 days; group K (n=6) control. Samples of intramuscular, visceral and subcutaneous fat, and *Longissimus dorsi* and *Vastus lateralis* were collected and subjected to qPCR. Several genes related to muscle and fat metabolism were investigated: myogenic regulatory factors, oxytocin/oxytocin receptor, atrophy and lipid metabolism genes.

Results: MYF6 was significantly up-regulated in group R in both the examined skeletal muscles, while Oxytocin gene was significantly up-regulated only in *Vastus lateralis*. Regarding adipose tissues, FABP4 was significantly up-regulated only in visceral fat. No statistical differences were observed between treated animals and controls in skeletal muscles for Atrogin, ADIPOR1 and ADIPOR2, ERα, FABP3 and FABP4, IGF1, Leptin receptor, MYH1 and MYH2, MYOG, PPARγ, nor Oxytocin receptor. Similarly, no differences were registered in the three adipose tissues for Adiponectin, IGF1, IL6, Leptin, Oxytocin and Oxytocin Receptor, PPARγ nor TNFα.

Conclusions: Novel biomarkers are needed to guarantee customer's safety in consideration of Transatlantic Trade and Investment Partnership approval (TTIP). Hormone implants are legally used in the USA and a screening test on imported meat will be auspicable. In previous studies Oxytocin and its pathway were suggested as promising biomarkers for estrogens. In this study, only slight differences between treated and control animals were detected, probably due to the presence of male steroid in Revalor-200.

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INFLUENCE OF EM-PROBIOTIC ON THE MORPHOLOGY OF THE LIVER AND GILLS IN ZANDER

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Introduction: The technology of effective microorganisms (EM) uses microorganisms to cooperate with the surrounding environment. Many research centers conduct study in order to reveal EM effects on aquatic organisms, including fish in aquaculture. The aim of the study was to evaluate effects of the EM-Probiotic (produced by Greenland) on the morphology of the liver and gills in zander (*Sander lucioperca*) reared in controlled conditions.

Materials and Methods: Fish were reared in recirculating aquaculture systems (RAS). The experimental feed (Aller) were administered to three groups of fish for 28 days - addition of EM was: 0% (control group - C), 2% and 4% of the weight of the feed. Zander gills and liver were collected for histopathological examination after 28 days of feeding with the EM-feed and two weeks later. Samples were fixed in 6% neutral buffered formalin, embedded in paraffin and stained with HE.

Results: During microscopic examination of the liver specimens significant differences in a degree of fatty degeneration were observed - small degree steatosis (C), from 30% to 100% of fatty tissue (2% EM) and the lack of changes (4% EM). Pathologic findings in gills were lamellar epithelial lifting (C), lamellar fusion (2% EM) and filament hyperplasia (2% EM).

Conclusions: The results indicate that EM can have an influence on the morphology of the liver and gills in zanders reared in RAS, depending on their amount in feed. There were also differences in the fish condition during the adaptation period, in favor of fish from the Group 4% EM.

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HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY OF *POLYSPOROPLASMA MUGILIS* (MYXOZOA) INFECTION IN KIDNEYS OF MULLETS (OSTEICHTHYES: MUGILIDAE) FROM SARDINIAN LAGOONS

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Introduction: *Polysporoplasma mugilis* (*Sphaerospora mugilis* n. comb.) are myxozoan histozoic parasites that infect kidney of mullets (Osteichthyes: Mugilidae). This study aimed to investigate the prevalence of granulomas caused by spores of *P.mugilis* and to characterise inflammatory response and granulomas evolutionary stages by histochemical and immunohistochemical techniques.

Materials and Methods: Mullet specimens (n=239) were sampled from Sardinian lagoons. Histological sections of kidney were stained with Hematoxylin-eosin, Masson's Trichrome and Periodic acid-Schiff. Epithelioid cells and fibroblasts were detected and quantified by immunohistochemistry [anti-cytokeratin (CK) AE1-AE3 and anti-Vimentin (Vim) antibodies] in order to characterise cellular components of granulomas. Rodlet cells (RCs) and eosinophilic granular cells (EGCs) were quantitatively evaluated by an open source image processing program (Rasband, W.S., ImageJ).

Results: At histological examination, 17% of mullets showed granulomas in glomeruli. The inflammatory response was significantly lower in early stage granulomas than in other stages ($P < 0.05$). Late stage granulomas showed EGCs aggregation and collagen component as prominent features ($P < 0.05$). Immunohistochemistry allowed to distinguish epithelioid cells (CK+) and fibroblasts (Vim+) that increased in intermediate and late stage granulomas, respectively ($P < 0.05$).

Conclusions: *Polysporoplasma mugilis* infection causes a mild to moderate renal tissue damage, leading to a partial functional loss of kidney tissue. This is the first report of histopathological and immunohistochemical features of granulomas due to *P.mugilis* in kidney of Mugilidae species.

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COMPARISON OF THE PATHOMORPHOLOGICAL EVALUATION OF THE GILLS AND LIVER IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS,* WALBAUM 1792) REARED BY TWO DIFFERENT TECHNOLOGIES

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Introduction: Rainbow trout is traditionally reared in extensive systems in the fresh water (1) but it is more and more often done using water recirculation (2), with maintaining animals welfare. The morphological examination of gills and liver is aimed at answering the question as to which of the organs is more prone to pathomorphological changes in each system.

Materials and Methods: The study was done on 960 rainbow trout in 48 groups (n =20) during two years, in spring and autumn, from 6 fish farms which are using the different rearing system – 1 (3 farms) or 2 (3 farms). The animals A had body weight of 350-500 g, and B had body weight of 501-850 g. Sections of gills and liver were stained with H&E and those of liver with PAS according to MacManus. The level of glicozaminoglicans was assessed semiquantitatively according to Szarek.

Results: Usually the structure of organs was normal. In gills elevation of epithelial cells, lamellar telangiectasis, aneurysms and hypertrophy of mucous cells dominated. In the liver (especially from animals 2B) steatosis simplex, congestion, melanomacrophages was seen, and sporadically parenchymatous degeneration, necrosis of singular hepatocytes and lymphocytic infiltration. More lesions were visible in gills than in the liver. They were more common in animals 2B in autumn. The level of glicozaminoglicans was higher in animals from farms 2, especially in autumn, and in animals B.

Conclusions: In gills the extensiveness of adaptational and alterative changes was higher than in liver, especially in the intensive rearing system in autumn in larger animals.

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INFLUENCE OF INFECTIOUS PANCREATIC NECROSIS VIRUS (IPNV) ON SURVIVAL RATE OF JUVENILE RAINBOW TROUT AFTER EXPERIMENTAL INFECTION

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Introduction: The aquatic infectious pancreatic necrosis virus (IPNV) causes a severe disease in farmed salmonid fish. IPNV has a very broad host range and infects many different species of fish as well as molluscs and crustaceans. Very little information exists about the influence of IPNV on the defence mechanisms in fish. In this study, we examine the effects of IPNV on the survival rate of rainbow trout (*Oncorhynchus mykiss*) after experimental infection with *Yersinia ruckeri*.

Materials and Methods: Two hundred healthy rainbow trouts and 200 asymptomatic IPN virus carriers were divided into four groups (100 fish per group) of a mean weight of 50g. One group of healthy fish and one group of carriers were inoculated intraperitoneally with 0.5 ml *Yersinia ruckeri* suspension $1x10^6$/ml. Fish from two other groups were inoculated intraperitoneally with 0.5 ml of PBS. Fish were observed for 10 days.

Results: Mortality in the IPNV carriers group reached 91% whereas the non-carriers group amounted to 53%. Fish mortality in the groups treated with PBS reached 12% and 1% respectively.

Conclusions: Asymptomatic IPNV carrier state is associated with increased susceptibility to infection with *Yersinia ruckeri* and much higher mortality. Therefore, the ability to make the right diagnosis of IPNV is important in breeding farms and should be performed routinely.

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OXIDATIVE STRESS BIOMARKERS IN MUSCLE TISSUE OF RAINBOW TROUT (ONCORHYNCHUS MYKISS WALBAUM) AFTER VACCINATION AGAINST *YERSINIA RUCKERI*

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Introduction: *Yersinia ruckeri* bacterin was the first commercially produced fish vaccine, and the formalin-killed whole-cell product continues to be highly effective. In the present study, we determined the influence of vaccination against yersiniosis on oxidative stress biomarkers and antioxidant defense in the muscle tissue of the rainbow trout (*Oncorhynchus mykiss*) vaccinated against *Y. ruckeri*.

Materials and Methods: The following parameters were evaluated in the muscles of rainbow trout immunised with *Y. ruckeri* vaccine: oxidative stress biomarkers – 2-thiobarbituric acid reactive substances (TBARS), aldehydic and ketonic derivatives of oxidatively modified proteins (OMB), as well as activities of the antioxidant enzymes. Healthy fish were vaccinated orally with inactivated whole cells of a virulent strain of *Y. ruckeri*. One and two months after immunisation the muscle samples were collected.

Results: No significant difference was noted in lipid peroxidation level. Aldehydic and ketonic derivatives of OMB in the vaccinated group were significantly lower in the second month compared to those in the first month after vaccination ($P < 0.05$). The content of ketonic derivatives of OMB in muscles in the first month after immunisation was higher compared to untreated control. All these culminated in a depletion of glutathione peroxidase (GPx) activity and low level of total antioxidant capacity (TAC).

Conclusions: It is suggested that immunisation of fish with *Yersinia* vaccine is associated with induced free radical formation and oxidative stress. Free radicals would therefore be at least partially responsible for the induction of both humoral and cellular elements of the immunity and increased protective immunity.

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THE INFLUENCE OF FEED SUPPLEMENTATION WITH KYNURENINE ACID (KYNA) ON THE GILLS IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) EXPERIMENTALLY INFECTED WITH *YERSINIA RUCKERI*

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

Materials and Methods: The experiment was conducted on 50rainbow trout. The animals were divided into 5 groups (n=10): control group injected 200 µl with PBS fed without any supplementation of KYNA, control group injected 200 µl of previously prepared *Y. ruckeri* (concentration 107/ml^{-1}) also fed without any supplementation of KYNA, and 3 experimental groups injected 200 µl *Y. ruckeri* and fed with suplementation of KYNA in doses: 2.5 mg/kg, 25 mg/kg and 250 mg/kg. From all fish, samples of gills were taken for histological examination.

Results: No changes were observed in the group with PBS. In the control group multifocal blood clots in blood vessels, multifocal accumulation of lymphoid cells, fusion, deformation and focal atrophy of lamella were observed. In the first research group destruction of cartilage, the presence of lymphoid cells between plaques and epithelial necrosis were found. In the second group additionally multifocal hyperplasia of epithelium was observed. Lesions in the group with the highest dose of KYNA were as follows: blood clots in the vessels, infiltration of lymphoid cells, epithelial necrosis, hyperplasia of goblet cells and cartilage destruction.

Conclusions: The results of histopathological examination raise concerns over the possibility of using KYNA as a feed supplement for rainbow trout.

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USE OF HISTOPATHOLOGICAL EXAMINATION IN HEALTH CONTROL OF CULTURED FISH – CASE REPORT

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Introduction: Regular health control of cultured fish using full spectrum of ichthyopathological diagnostic methods is not a common practice. Usually the determinant of good health is the absence of visible clinical symptoms. The moment when changes are visible often occurs too late to stop and reverse the effects of pathological processes in the fish organism. The aim of the study was to analyze the usefulness of histopathologic techniques in control examination of apparently healthy fish.

Materials and Methods: A total of n=7 fish hybrids of red and silver strains of Nile tilapia *Oreochromis niloticus* without any clinical symptoms were examined. Fish underwent a routine procedure of clinical examination. Samples from the liver, anterior and posterior kidney were collected for histopathological examination during necropsy. Material was fixed in 6% neutral buffered formalin, subsequently dehydrated, embedded in paraffin and stained with H&E. Afterwards specimens were analyzed by light microscopy and evaluated.

Results: Histopathological analysis revealed severe pathomorphological lesions in the liver, mostly steatosis of significant degree, fibrosis and distorted structure of the tissue. Most likely they were caused by inappropriate diet. The detection of changes led to modifications in nutritional regime.

Conclusions: From the above case we can infer that regular performing of complex examinations is requisite for maintaining health of farmed fish. Diagnostic pathomorphology and necropsy facilitates early detection of morphological lesions in the internal organs, which allows for quick treatment and adjustment of the farming conditions for preventing stock losses.

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STURGEON NUCLEO-CYTOPLASMIC LARGE DNA VIRUS AND ACINETOBACTER SPP. CO-INFECTION: PATHOLOGICAL FINDINGS IN A DISEASE OUTBREAK

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Introduction: Sturgeon farming is increasing in several European countries, however its success is heavily limited due to the paucity of information related to the diseases that can affect sturgeons. The aim of the present work is to describe the pathological findings in a disease outbreak which occurred in farmed juveniles of *Acipenser gueldenstaedtii* and *A. baerii*.

Materials and Methods: Samples of tissues from fish showing clinical signs as well as recovered animals were routinely processed for histology. Samples of skin and gills were also submitted for ultrastructural examination. Bacteriological examinations and molecular detection for nucleo-cytoplasmic large DNA virus (NCDLV) were carried out.

Results: Multifocal ulceration and reddening of integument and fins were the main gross findings. The gills were affected by circulatory disturbances and showed lamellar hyperplasia. Histologically the integument and the fins were ulcerated or hyperplastic, the gills were moderately hyperplastic and in some cases showed necrosis of pillar cells. Both in skin and gill tissues a few hypertrophic, cytomegalic epithelial cells with homogenous amphophilic cytoplasmic inclusions were detected. A virus similar to North American sturgeon NCDLVs was detected in diseased and recovered fish. Bacteriological examination and molecular identification revealed the presence of *Acinetobacter* spp. and *Aeromonas* spp. in fish with clinical signs.

Conclusions: The presence of a sturgeon NCLDV was interpreted as the primary cause of disease, while the bacteria *Acinetobacter* spp. and *Aeromonas* spp. were considered as concurrent infections. However for *Acinetobacter* spp. an active role in causing the disease outbreak was suspected.

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HISTOPATHOLOGICAL FINDINGS IN THE CENTRAL NERVOUS SYSTEM OF MONTEZUMA SWORDTAIL (*XIPHOPHORUS MONTEZUMAE* JORDAN & SNYDER, 1899) FRY WITH EARLY MORTALITY ASSOCIATED WITH SEVERE NEUROLOGICAL SIGNS

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Introduction: Neurological syndromes in fry may be associated with internal or external hydrocephalus caused by a variety of injuries, including toxic, nutritional, infectious, and genetic/heritable factors. However, limited information concerning histopathological features of nervous tissue of fish with neurological signs is available.

Materials and Methods: A total of 33 live, swim-up fry were produced in an aquarium colony of the live-bearer *Xiphophorus montezumae*, composed by 3 females and 1 male. About fifteen days after birth, fry showed severe neurological signs ranging from anorexia and lethargy to swimming difficulties, whirling and loss of equilibrium. No gross abnormality was evident. Immediately after spontaneous death, 31 fry were formalin-fixed and routinely processed for histology. Two fry survived without clinical signs. Adult fish were not affected.

Results: Histological examination of all affected fry revealed various degrees of external hydrocephalus characterized by increased space between the meninges and the brain, with presence of scant protein-like material in the sub-arachnoid space. Variable, usually mild, vacuolation of the nervous tissue was also observed. Brain was only covered by the skin on the dorsal part of chondrocranium.

Conclusions: This is the first description of neurological disease associated with histological features of external hydrocephalus in the Montezuma swordtail. Water quality analysis and absence of pharmacological treatments allowed to exclude toxic causes. Vitamin deficiencies were also unlikely, since a well-balanced and integrated, commercial diet specific for swim-up fry was provided. Based upon the lack of evidence for an infectious, especially viral, etiology, a congenital, possibly genetic, condition may be supposed.

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HISTOPATHOLOGICAL AND IMMUNE-RELATED ASPECTS OF KOI HERPESVIRUS INFECTION IN CARPS

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Introduction: Koi herpesvirus (KHV, CyHV-3), like the other members of the *Herpesviridae* family, shows ability to establish life-long infection in immunocompetent hosts. To get more knowledge on the immune evasion strategy exploited by KHV, we infected carp fry with KHV and measured the expression of host MHC class I in tissues of internal organs of fish.

Materials and Methods: Tissue sections of kidney, gills, brain, gut and liver were collected when clinical signs of the disease were evident and snap frozen for subsequent RNA extraction or fixed in 10% buffered formalin for histopathological examination. For assessment of MHC I mRNA expression, a quantitative real-time PCR targeting the KHV thymidine kinase gene and fragment of the MHC I sequence was developed.

Results: We found that the expression of the MHC I in infected tissues of all studied organs was significantly reduced but spleen compared well to uninfected tissues. The highest differences were observed in the intestinum of infected carps while in the spleen no statistically significant differences were found. The differences in MHC I expression in the remaining organs were roughly inversely correlated with the virus load. Infected fish showed a variety of histopathological changes, among them presence of cellular inflammatory infiltrates, degeneration of tubular epithelium in the nephrons and congestion of the vessels were the most prominent.

Conclusions: In summary, we confirmed our hypothesis that KHV infection results in down-regulation of MHC I expression in infected tissues and the extent of this immunosuppressive effect is roughly inversely correlated with the virus load.

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EXPERIMENTAL BRUISES IN PIGS

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Introduction: Determining the age of bruises is a challenge in forensic pathology. In pigs, more than 90% of bruises are assumed to be inflicted within 8 hours before slaughter in which period pigs are handled by more people. To determine in whose custody the pig was when bruises were inflicted a determination of the age is crucial. The aim of the study was to develop a porcine model of bruises and to study the changes in the lesions over time.

Materials and Methods: Ten female pigs were anesthetized and four blunt traumas were inflicted on the back using a steel construction. Pigs were kept in anaesthesia and euthanized from 1 to 10 h after the bruises had been inflicted. The bruises were then subjected to gross and histological evaluation. Normal skin and muscle tissue were sampled from two control pigs.

Results: Grossly, bruises were uniform and consisted of two parallel lines of haemorrhage in the skin. Histologically, haemorrhages and leukocytes were seen in the subcutaneous tissue and the underlying muscle tissue. In addition, in the muscle tissue necrotic muscle fibres were observed. The number of neutrophils within the subcutaneous tissue and the number of macrophages infiltrating muscle tissue increased with increasing age of bruises. Moreover, the localization of leukocytes in the muscle tissue showed a time dependent response.

Conclusions: Histological evaluation of bruises was able to determine the age of bruises as being more or less than 4 h. Gross evaluation of bruises could not be used for age determination.

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CRANIOMETRY IS A USEFUL TOOL FOR FORENSIC DIAGNOSTIC PATHOLOGY IN NEOTROPICAL PRIMATES

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Introduction: Craniometry, or craniology, is the study of cranial metric parameters that generate data, in a universal way, that allow identification of characteristics such as species, ethnics, race, age and sex. Since little is known about cranial structure of various wild species, data obtained from domestic animals is frequently extrapolated, resulting in a poor model for accurate forensic routine in wild animals, particularly tropical monkeys.

Materials and Methods: Craniometric points for nine capuchin (*Sapajus libidinosus*) and six howler (*Alouatta caraya*) monkeys were established through a comparative study with previously documented human craniometry. Specimens were categorized into five age groups (infants, young, sub-adults, adults and old), according to dental parameters and basisphenoid and basioccipital synostosis. With the aid of calipers and a set square, 15 measurements were established to differentiate the two species.

Results: Despite evolutionary-related anatomical differences, most of the 52 points described for humans were observed in both capuchins and howler monkeys, and such similarities warranted the comparison between species. The points named ALVEOLON, LINGULARE, MEXILLOFRONTALE, EKTOKONCHION e SUBSPINALLE were absent in both monkey species, whereas the STENION point was absent in *A. caraya*. Six sub-adults and three adults of *S. libidinosus*, and one old and five sub-adults of *A. caraya* were identified. The jaw articular process height and the mandibular length were statistically longer in *A. caraya*.

Conclusions: Determination of morphological differences and craniometrical points of *Sapajus libidinosus* and *Alouatta caraya* specimens allows positive recognition of individuals from these species, providing useful information for forensic diagnostic pathology.

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SERIAL OBSERVATIONS ON POSTMORTEM CHANGES OF PORCINE SKELETAL MUSCLES AFFECTED BY BLOWFLY MAGGOTS

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Introduction: The estimation of postmortem interval is one of the primary works in solving crime cases. However, limited methods were applied in forensic fieldwork until now. Given that the postmortem changes are dramatically influenced by numerous intrinsic and extrinsic factors, the better way to approach practical methods for estimating postmortem interval is to perform factor based observation of postmortem changes. The present study aims to investigate the postmortem changes of skeletal muscles under the influence of maggots.

Materials and Methods: Histology and immunohistochemistry based methods were applied to observe and to quantify the morphological changes and proteolysis of skeletal muscle cells with or without maggot (*Chrysomya megacephala*) involvement.

Results: The results demonstrated that under maggot influence, physical destructions took place between maggots and debris of muscle tissues and the adjacent remaining myocytes underwent chemical destructions. Besides, the immunohistochemistry against dystrophin, desmin, and myoglobin showed that positive signals faded out with time faster in groups with maggot effect than the control groups.

Conclusions: Under maggot effect, the acceleration of postmortem changes can be illustrated by means of immunohistochemistry. Proteolysis profiles may be served as a potential tool for estimating postmortem interval.

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CEREBELLAR HISTOMORPHOMETRY FOR AGE DETERMINATION OF PUPPIES IN VETERINARY FORENSIC PATHOLOGY

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Introduction: In diagnostic cases of legal relevance, age determination of puppies is obtained by documentation of degree of physiological maturation of some morphological processes. Development of cerebellar cortex is one among the maturative processes that continues after birth. Our aim is to quantify, via digital image analysis, the cerebellar cortical maturation in 0-75 days old puppies, investigating the suitability of this parameter as ancillary tool for age determination for forensic purposes.

Material and Methods: Thirty puppies of nineteen breeds were divided into three age classes (days): A: 0-25; B: 26-50; C: 51-75. Thickness of both external granular layer (EGLT) and molecular layer (MLT), their ratio (EGLT/MLT) and the number of cell layers composing the EGL (cellular thickness) were evaluated under light microscopy and measured using image analysis software (ImageJ).

Results: Both the correlations between age and EGLT/MLT (Spearman R=-0.8464; p=0.0000) and between age and EGL cellular thickness (Spearman R=-0.7968; p=0.0000) were negative and significant.

Conclusions: The thinning of the EGL and synchronous thickening of the molecular layer are sensitive morphological parameters for determination of cerebellar maturation in puppies. Therefore evaluation of EGLT/MLT and EGL cellular thickness can be useful for forensic purposes.

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83 HISTOPATHOLOGY AND QUANTITATIVE PATHOLOGY OF SKELETAL MUSCLE ATROPHY IN STARVED DOGS: RELEVANT FORENSIC ORIENTED FINDINGS

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Introduction: Fatal starvation is often encountered in veterinary forensic pathology and this entity may become of major medicolegal importance if death results from deliberate withholding of food in domestic animals. Following food deprivation, a progressive mobilization of skeletal muscle proteins as a source of energy occurs and lasts until the animal's death. The aim of the present pilot study is to describe the histological and morphometric changes of skeletal muscle in starved dogs.

Materials and Methods: The database of Veterinary Pathology-School of Veterinary Science-University of Liverpool, was searched for dogs (expected body weight over 15 kg and more than one year of age) in which starvation was identified as "cause of death". H&E stained slides of quadriceps muscles from 13 starved and seven control animals were evaluated for histopathological changes. Morphometry of 168±58 myofibres per quadriceps was performed to measure the minimal Feret's diameter.

Results: In starved dogs, prominence of subsarcolemmal nuclei with nuclear clumping and decrease and variation of myofibre diameter were significantly higher when compared to controls ($p<0.05$). Oedema and fibrosis were not associated with starvation ($p>0.05$). Difference in mean Feret's diameter (21.89±5.16 μ starved, 39.68±3.05 μ control) was also significant ($p<0.05$).

Conclusions: The prominence of subsarcolemmal nuclei with nuclear clumping, decrease and variation of myofibre diameter in absence of denervation pattern and other degenerative changes were typical histological features of starvation-induced muscular atrophy of quadriceps muscle in dogs.

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DISTRIBUTION OF ADIPOCYTE MITOCONDRIA IN *BOS TAURUS*

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Introduction: Mitochondria plays a crucial role in the energy homeostasis of the body. An important aspect in understanding the dynamics behind the metabolic regulation is the distribution of mitochondrial populations in the fat tissue. The aim of our study was to establish a correlation between the size and the number of mitochondria of white adipocytes for different Body Condition Scores (BCS).

Materials and Methods: In this study, three groups of cows have been used: 1) cachectic, 2) normal weight, and the 3) overweight group. Adipose tissue samples (1mm^3) from the subcutaneous region and the kidney area have been collected for electron microscopy analysis of mitochondria.

Results: In this study 678 mitochondria have been measured. Overall, the average length of mitochondria in cow adipocytes showed a value of 0.64 µm (SD ± 0.41) and the average widths of mitochondria showed a value of 0.25 µm (± SD 0.09). The main observation consists of a distribution void of "forbidden" mitochondrial size in which no mitochondrion in adipocytes is within the range of lengths of 0.7 µm - 1.2 µm and widths of 0.4 µm - 0.6 µm.

Conclusions: The maximum average volume of mitochondria observed in this study was found in cattle of normal weight. Interestingly, the average volume of mitochondria in overweight cattle adipocytes is less than the average volume of mitochondria in normal weight cattle adipocytes, which suggests that the physiological state and / or mechanical stress exerted by the lipid droplets in obesity reduces the volume of adipocytes mitochondria.

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A CRYPTIC CASE OF CANINE CRYPTOCOCCOSIS IN IRELAND

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Introduction: Cryptococcosis caused by *Cryptococcus neoformans* or *C. gattii*, is an important mycotic disease in immunosuppressed hosts and affects many mammalian species worldwide. However, it is rarely reported in Ireland. The characteristic histologic lesion is a mass of encapsulated yeasts with mild granulomatous inflammatory reaction mainly causing rhinitis, meningitis and dermatitis. Occasionally, dissemination to the lung and other organs occurs.

Materials and Methods: A 2.5-year-old female Springer Spaniel presented to the University Veterinary Hospital Dublin with a 12 day history of a subcutaneous mass on the neck (pyogranulomatous panniculitis), inappetence and pyrexia (41°C). Radiographs showed multifocal pulmonary nodules. The dog died despite treatment.

Results: Post mortem examination identified the mass on the neck as severe granulomatous lymphadenitis. Multifocal widespread granulomas were further seen in the lungs and in the nasal cavity. All lesions were associated with fibrinous vasculitis and necrosis. No aetiological agent could be detected on H&E and numerous histochemical stains. Microbiological tissue cultures were negative. Subsequent Fluorescence in situ hybridization (FISH) revealed multifocal clusters of fungi identified as *C. neoformans*. PCR and sequencing results will follow.

Conclusions: This is an unusual presentation of a sporadic disease in a dog. The widespread granulomas associated with vasculitis and marked tissue damage in the absence of the characteristic large numbers of encapsulated yeasts in an immunocompetent host suggest that the disease was caused by an overexuberant inflammatory reaction. *C. neoformans* should be considered as a differential diagnosis in subcutaneous granulomas, where no infectious agent is detectable by routine histological methods.

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A CASE OF SYSTEMIC CANINE PROTOTHECOSIS IN ITALY

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Introduction: Protothecosis is a rare disease of human and animals caused by aerobic, microscopic, unicellular, achlorophyllic algae of the genus Prototheca, family Chlorellaceae. The canine infections tend to be more severe, with systemic diffusion of the disease. In Italy prototheca mastitis in cattle is well known but very few cases of canine protothecosis have been reported.

Materials and Methods: A 2-year-old, male mixed-breed dog was referred with a 30 days history of poorly localized pain and neurological signs. Magnetic resonance imaging revealed multifocal lesions compatible with meningoencephalitis . In the three weeks following MRI the neurologic signs remained stable but the dog developed anorexia and vomiting. An acute renal insufficiency was diagnosed. Because of this severe complication, the dog was euthanized upon request of the owner and a complete post mortem examination was performed.

Results: Post-mortem examination and histopathological evalutation revealed multiple disseminated inflammatory foci associated with algae-like structures with cell walls positive with periodic-acid-Schiff (PAS) and metenamina silver stain. Morphological features were consistent with Prototheca.

Conclusions: Protothecosis should always be included in the list of differential diagnoses of an animal with signs of multifocal lesions of the CNS, especially when an inflammatory process affecting multiple organs is present.

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87 INFECTED RESERVOIR HOSTS SUGGEST A NOVEL ENDEMIC AREA FOR BORNA DISEASE IN AUSTRIA

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Introduction: In 2015 two cases of Borna disease were diagnosed in horses from Upper Austria. The cases occurred within a short time period and the distance of the affected stables was only 5 km. In order to show whether the causative agent was also harbored by its reservoir host, the bicolored white-toothed shrew (*Crocidura leucodon*), 28 shrews from this geographic area were collected and investigated for presence of Borna disease virus-1 (BoDV-1).

Materials and Methods: The shrew species were identified according to taxonomic clues and molecular barcodes, and investigated by immunohistochemistry (IHC) and RT-PCR, respectively. Amplification products were subjected to sequencing and the acquired sequences were compared to GenBank entries of BoDV as well as to unpublished sequence data.

Results: Among the 28 shrews, 9 were identified as *C. leucodon* and 13 as *Sorex araneus*. Six *C. leucodon* (66.7%) and one *S. araneus* (7.7%) had BoDV-1 infections. In accordance with previous findings, IHC of *C. leucodon* showed abundant amounts of viral antigens in many neural and extraneural tissues. In contrast, the single positive *S. araneus* had an exclusively neural staining pattern. The acquired sequences were not identical to each other, but clustered around the sequences of the horses with BD from the region.

Conclusions: These findings underline that the BoDV lineages show a strict regional genetic clustering which is related to the territorially bound reservoir hosts. Further, this is the first description of a BoDV infection in a shrew species other than *C. leucodon,* probably due to a spill-over event.

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ECHINOCOCCUS EQUINUS IN A HORSE: A RISK WHEN RAW-MEAT IS FED TO DOGS?

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Introduction: *Echinococcus (E.) equinus* is a cestode with a 2-host life cycle, with carnivores as definitive hosts, shedding eggs with the feces, and equines as intermediate hosts with hydatid cysts forming mainly in the liver and lung.

Materials and Methods: A 17-year old Connemara horse with an oromaxillary sinus fistula and chronic colitis was euthanized with poor prognosis. The horse had a history of long-term corticosteroid-therapy. Necropsy identified several well-demarcated, grey-white cysts of up to 5 cm in diameter in the liver parenchyma, filled with clear, amber-colored liquid containing particles of less than 1 mm in diameter (hydatid sand). These cysts were further investigated by histopathology and polymerase chain reaction (PCR).

Results: At necropsy, additional smaller cysts of up to 1 cm in diameter were visible in the liver and also in the lung. Histopathology revealed a tri-layered appearance of the cysts with an outer connective tissue capsule followed by a PAS-positive, hyaline acellular layer and an inner germinal membrane. The cysts contained few free protoscolices indicating the presence of *Echinococcus* spp. PCR product sequencing revealed complete identity with *E. equinus* 12S rRNA and Cytochromoxidase 1.

Conclusion: The present case indicates that although *E. equinus* infections are still considered rare, they have emerged to our previously unaffected latitude. They pose a potential risk with dogs being fed with raw horse-meat or –liver in terms of the BARF (Biologically Appropriate Raw Foods)-diet which has gained increasing popularity. These dogs may potentially become reservoirs for *E. equinus*, a risk which should be considered.

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DETECTION OF MAEDI-VISNA ANTIGEN IN MAMMARY GLAND AND MILK BY IMMUNOHISTOCHEMISTRY

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Introduction: Macrophages are the main target cells of ovine Maedi Visna virus (MVV), although other cells such as epithelial cells have been suggested as possible targets of the disease. The aim of this study is to identify the target MVV cells in mammary gland and its implication in Maedi Visna (MV) transmission through lactation in natural cases.

Materials and methods: Mammary gland samples of 19 naturally-infected sheep and 10 negative controls from intensive milk-producing flocks and milk samples from tank container, seropositive and seronegative animals were studied. Immunohistochemistry and immunocytochemistry against gp135 and p28 of MVV were carried out.

Results: MV infection was confirmed in mammary gland always associated with lesions, even minimal and focal. Macrophages were the only positive cells observed, located in the interstitium (n=19), acinar lumen (n=2) and milk samples. No positivity was found in epithelial cells.

Conclusions: The presence of MVV antigen in mammary gland and milk confirms the potential transmission of the disease through lactation. The presence of this virus was only associated with inflammatory cells, confirming the hypothesis of viral invasion via monocytes/macrophages which would begin in very focally concrete locations and then spread to the rest of the tissue. No positivity was observed in epithelial cells in contrast to the hypothesis of the possible MV replication in these cells as has been described *in vitro*.

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ANTIFUNGAL SUSCEPTIBILITY OF CANINE AND FELINE *MALASSEZIA* SPP. ISOLATES TO LACTOFERRICIN: IN VITRO PRELIMINARY STUDY

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Introduction: *Malassezia* spp. is a fungus isolated from skin and mucosae of dogs and cats, that may cause dermatitis and otitis. Therapies include the use of antibiotics, antifungals and glucocorticoids. Because of the antibiotic-resistance phenomenon new alternative therapies are necessary. Bovine lactoferricin (Lfc) is a peptide derived from proteolytic cleavage of lactoferrin with proved antibacterial, antifungal and immunostimulatory activity. Aim of the study was to evaluate the antifungal susceptibility of *Malassezia* spp. to Lfc-Candioli Pharma (water solution 20%) using a microdilution method.

Materials and Methods: Fifty strains of *Malassezia* spp. collected from 50 animals (5 cats, 45 dogs) affected by dermatitis and/or externa otitis classified based on clinical signs and/or skin biopsies were cultured on Sabouraud dextrose broth. Minimal inhibitory concentrations of Lfc were measured using the following concentrations: 13.3%, 10%, 6.7 %, 3.3%, 1.8%. Plates were incubated at 35°C and read four days after inoculation. To check the reproducibility of the procedure all the isolates were double tested and quality controls were performed.

Results: All isolates were inhibited by Lfc with different minimum inhibitory concentration value. Product showed antifungal efficacy of 100% up to a dilution corresponding to 10% of Lfc. The first resistance was observed from 6.7% to the total resistance of 1.8%.

Conclusions: These results suggest a potential antifungal in vivo efficacy of Lfc, even if in vitro data should be considered with caution until standardized methods and correlation with clinical outcomes has been evaluated .

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BESNOITIOSIS IN AN EUROPEAN DONKEY

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Introduction: A 3-year-old castrated male donkey was presented with multifocal, moderately firm, dull-white nodules, varying in size from 0.5-2.0 cm in diameter. The nodules were located on the penile shaft skin and showed depigmentation. Other locations on the body were not involved.

Materials and Methods: Surgical excision of one large nodule and multiple smaller nodules was performed. All formalin-fixed samples were embedded in paraffin and routinely processed for histopathological examination.

Results: Histopathological examination revealed presence of multifocal, round to oval, protozoal cysts within the dermis, with a size of 150-500 µm. The mature cyst walls consisted of four distinct layers, including an outer, hyalinized, eosinophilic layer of collagen fibers, a thin homogenous intermediate layer, a layer consisting of the cytoplasm of the host fibroblast with a compressed nucleus, and an inner layer that formed the parasitophorous vacuole. The vacuole was filled with numerous bradyzoites of 2 x 8 µm. There was a mild to moderate, superficial to mid-dermal infiltration of lymphocytes, plasma cells, macrophages and eosinophils, surrounding the cysts and blood vessels. The hair follicles were atrophic. The overlying epidermis showed mild acanthosis and orthokeratotic hyperkeratosis. Based on the histopathological findings, a diagnosis of Besnoitia sp. infection was made. The large nodule was diagnosed as a sarcoid (early stage).

Conclusions: Besnoitiosis is an emerging disease in cattle in Europe. Few outbreaks have been reported in donkeys in the USA. To our knowledge, this is the first report in the literature of Besnoitia sp. infection in a European donkey.

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SYSTEMIC PROTOTHECOSIS IN A DOG

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Introduction: Protothecosis is an uncommon disease caused by algae of the genus *Prototheca*. Infections due to *Prototheca* spp. have been reported in humans as well as in domestic and wild animals. Furthermore, *P. zopfii* has presented in disseminated forms in dogs.

Materials and Methods: A 30 months-old, male, mixed breed dog was referred to the Animal Hospital of the University of Teramo with a history of chronic diarrhoea and severe neurological signs. Clinically, chorioretinitis, uveitis and a central vestibular syndrome were observed, with the dog subsequently developing a cluster of seizures and dying some days after hospitalization. A complete necropsy was performed at IZSAM "G. Caporale". In-depth histopathological, histochemical, immunohistochemical and microbiological investigations were carried out from a range of tissues.

Results: Macroscopically, disseminated, white-grayish, nodular and necrotic lesions were seen affecting the brain parenchyma, along with the atrio-ventricular endocardium and myocardium and both kidneys. A severe colitis was also apparent. Microscopically, multiple aggregates of PAS-positive, algal-like bodies, neighboured or not by a mainly histio-plasmacytic reaction, were observed in brain, heart, kidney, gut and spleen. Cultures from the aforementioned tissues yielded *P. zopfii* isolation.

Conclusions: The *intra vitam* diagnosis of protothecosis is complicated by the fact that several other *noxae* may cause diarrhoea in dogs. Furthermore, an under estimation of the real prevalence of such infection is reasonably justified by the fact that *Prototheca* spp. is not generally included in the differential diagnosis protocol, unless a systemic infection develops, as in the case reported here.

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FOLLOW-UP STUDY IN SHEEP EXPERIMENTALLY EXPOSED TO MAEDI-VISNA VIRUS

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Introduction: Maedi-Visna virus (MVV) causes chronic inflammatory changes in lung, central nervous system (CNS), udder and joints of sheep. Herein, we studied whether the neurovirulence of one MVV ovine isolate was preserved after experimental passage into sheep.

Materials and Methods: Firstly, MVV subtype B3 was isolated from sheep affected by clinical leucoencephalomyelitis and non-clinically manifested pneumonia and mastitis. MVV isolate was inoculated intravenously and intratracheally in lambs (n.10), which were housed together with other 2 non-inoculated ones. A clinical, serological and virological long-term follow-up was performed on these animals before being serially culled at 500 (n. 2), 700 (n. 2), 900 (n. 3), and 1100 (n. 5) days *post- inoculum* (*p.i*.).

Results: At 13 days *p.i.* MVV DNA was detected by PCR in 5 of the 10 lambs, whilst antibodies were evident by ELISA at 21 days in 6 of the 10 lambs. Lymphoproliferative lesions were observed in the mammary glands and in the lungs from 9 of the inoculated animals. In all the sheep, MVV DNA was detected in the lungs and mammary glands with lymphoproliferative lesions, except in the 2 sheep sacrificed at 500 *p.i.*. No evidences of infection were observed in the CNS. Interestingly, non- inoculated lambs contained MVV DNA in the blood at 105 days following the exposure, while lesions related to MVV were detected at 1100 days *p.i.*.

Conclusions: Our results demonstrate that unknown factors are crucial for inducing leucoencephalomyelitis after experimental infection with MVV subtype B3, while mastitis and pneumonia are more easily reproducible.

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94 IMMUNE CELL PROFILE IN MICE ORALLY INFECTED WITH *ENCEPHALITOZOON CUNICULI*

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Introduction: Microsporidia are intracellular pathogens causing self-limited and severe diseases in immunocompetent and immunocompromised individuals, respectively. Although infection by microsporidia is mostly acquired by the oral route, to date most studies about the immune response used the intraperitoneal (ip) route of infection. The aim of this study was to evaluate the immunity against *Encephalitozoon cuniculi* inoculated orally.

Materials and Methods: BALB/c, BALB/c XID (B-1 cells deficient, called XID) and XID adaptively transferred with B-1 cells (XID+B-1) were orally infected with *E. cuniculi* spores. After 21 days post-infection, spleen, Peyer's patches and peritoneal cells were evaluated by flow cytometry.

Results: Populations of peritoneal macrophages increased significantly in infected BALB/c mice compared to controls, but in XID and XID+B-1 mice this difference were not observed. B-1 cells, CD4+ and CD8+ T lymphocytes decreased only in peritoneal cavity from XID+B-1 infected animals. It was not observed the same in both peritoneal cavity from BALB/c and XID animals. The spleen´s analysis showed decrease of T lymphocytes in all infected groups. The amount of dendritic cells of Peyer's patch showed no differences between infected or uninfected animals.

Conclusions: These results suggest that the absence of B-1 cells negatively affects the recruitment of peritoneal macrophages. We observed decline in splenic $CD4^+$ and $CD8^+$ T lymphocytes populations from infected mice, so we speculate and investigate whether these lymphocytes may be being recruited to the infection site.

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SCHMALLENBERG VIRUS: PATHOLOGICAL AND VIROLOGICAL FINDINGS IN AFFECTED SHEEP FLOCKS

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Introduction: Schmallenberg virus (SBV) has recently been identified as the causative agent of congenital malformations in stillborn ruminants in Europe. Herein, we report the findings of a long-term follow-up study in Sardinian sheep flocks affected by SBV.

Materials and Methods: A number of SBV affected flocks were submitted to a long term follow-up study, during which we monitored the evolution of the disease by serological, virological and anatomo-histo-pathological investigations.

Results: Within-flock seroprevalence ranged from 23.9% to 73.8%, while the incidence of malformed lambs varied between 3% and 19%. In one flock the incidence of malformations in ewes with twin pregnancy was significantly higher (88%) than in ewes carrying a single pregnancy (4%). A significantly higher occurrence of malformations (92%) involved only one of the twin lambs . The morphological examination of 105 malformed lambs mostly found brachygnathia (75%), arthrogryposis (86%), curvature of the vertebral spine (62%), hydrocephalus (26%), cerebellar hypoplasia (43%) and rare spinal cord hypoplasia. Histologically, moderate focal non-purulent inflammatory changes were observed in some malformed brains with T lymphocytesbeing the most represented inflammatory cells. Only 75% of the malformed lambs were positive to SBV by RT- PCR. Sequencing demonstrated that Sardinian SBV isolates did not display statistically significant differences in the genoma, when compared to other European isolates.

Conclusions: The clinical-pathological aspects of SBV infection encountered in this study were similar to those that have already been observed by others in Europe, and to those attributed to Akabane virus as well as to other viruses of the Simbu group.

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PHAGOCYTIC INDEX OF *MALASSEZIA PACHYDERMATIS* AND *MALASSEZIA FURFUR* BY A MURINE MACROPHAGE CELL LINE - AN IN VITRO STUDY

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Introduction: Yeasts of the genus *Malassezia* are part of the normal cutaneous microbiome. They live in an interface between commensal and pathogen and in immunocompromised patients, *M. furfur* and *M. pachydermatis* may be associated with fungemia and sepsis. Phagocytosis is an important nonspecific immune mechanism, but there is limited information about phagocytosis index and killing of *Malassezia* spp. Thus, the aim of this work was to determine the phagocytosis index of *M. furfur* and *M. pachydermatis* by macrophage cell line RAW 264.7.

Material and Methods: Murine RAW-264.7 macrophages were infected with *M. furfur* CBS-1878 or *M. pachydermatis* CBS-1696 in increasing ratios 1:0.5, 1:2 and 1:5 (macrophages/yeast), respectively, and incubated at 37°C with 5% CO_2. After 30 min, cover glass were collected and submitted to Giemsa stained. Phagocytic index was calculated considering % of macrophages that internalized at least one yeast X the average number of fungal cells in these macrophages.

Results: The average uptake of *M. furfur* and *M. pachydermatis* in the ratios 0.5:1, 2:1, 5:1 were 108, 67 and 28 vs 122, 70 and 22 respectively. Phagocytosis index for *M. furfur* and *M. pachydermatis* were 172.8, 108.0 and 39.3 vs 244.0, 101.5 and 30.8 respectively. Phagocytic index was statistically at 5:1 ratio ($p<0.05$), but no differences were observed between these *Malassezia* species in relation to phagocytic activity.

Conclusions: We conclude the ratio 5:1 (yeasts/macrophage) was the most suitable for the analysis of phagocytosis index of *M. furfur* and *M. pachydermatis* by RAW 264.7 macrophages in an in vitro assay.

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B-1 CELL INFLUENCE ON MICROBICIDAL ACTIVITY OF MURINE MACROPHAGES IN AN *IN VITRO* ENCEPHALITOZOONOSIS MODEL

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Introduction: Recently, it was demonstrated that B-1 cells can down-regulate the efficacy of macrophages to eliminate pathogens by reduction of nitric oxide (NO) production. *Encephalitozoon cuniculi* is an opportunist intracellular pathogen of mammals. As macrophages play an important role presenting and killing pathogens, this study aims to demonstrate the influence of the B-1 cell in the macrophage activity in encephalitozoonosis.

Materials and Methods: NO production was measured as nitrite form by adding Griess reagent in the supernatants of (1) Adherent Peritoneal Cells (APerC) from BALB/c mice (with macrophages and B-1 cells), (2) APerC from XID (B-1 cells deficient) mice, and (3) macrophages cultures (ATCC Raw 264.7 cell line) infected or not with *E. cuniculi* after 30 minutes, 1, 48, 96 and 144 hours post cells infection. In addition, the phagocytic index was calculated.

Results: Levels of NO in supernatants of APerC from XID mice (96 and 144 hours) and macrophages cultures (1 and 144 hours) infected with *E. cuniculi* were significantly higher than uninfected culture. However, the levels of NO in APerC from BALB/c mice inoculated with *E. cuniculi* were not different of uninfected control culture. Additionally, the phagocytic index of APerC from XID mice (0.40) and macrophages (0.45) cultures was significantly higher than that of APerC from BALB/c mice (0.11).

Conclusions: Together, these data suggest that B-1 cells induce down-regulation of macrophage microbicidal activity in an *in vitro* murine encephalitozoonosis model.

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INTESTINAL HISTOPATHOLOGICAL PATTERN ASSOCIATED WITH INFECTION BY *ENCEPHALITOZOON INTESTINALIS*

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Introduction: Microsporidia are obligate intracellular pathogens that infect many vertebrate and invertebrate hosts. *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* are most identified in human microsporidia as diarrhea agents; however the evolution and progression of the infection and the pathological changes are still poorly studied. We demonstrated patterns of lesions observed in mice immunosuppressed with cyclophosphamide or not and infected with *E. intestinalis*.

Materials and Methods: C57BL/6 mice, immunosuppressed with cyclophosphamide or not, were inoculated orally with *E. intestinalis* spores. Animals were euthanized at 7, 14, 21 and 28 days post-infection (DPI) and intestines samples were collected, fixed in 10% buffered formalin solution, prepared for histological analysis, and stained with Hematoxylin-Eosin and Gram-Chromotrope stain.

Results: We observed progression of the lesions and the parasite burden between 7 and 14 DPI, followed by reduction of these at 21 and 28 DPI. In the duodenum of the non-immunosuppressed mice lymphoplasmocytic enteritis with areas of apical necrosis, and a low frequency of vacuoles with *E. intestinalis* spores was noted. In the ileum, we observed a similar infiltrate but an intense presence of vacuoles with spores, mainly in glandular region. The Peyer's patches showed a mixed pattern hyperplasia. In immunosuppressed mice there was disclosure of large parasite burden, especially in the ileum, but infiltrates were scarce and Peyer's patches were rarefied.

Conclusions: The intestinal lesions characteristic of oral infection by *E. intestinalis* developed between 7 and 14 DPI and gradually decreased between 21 and 28 days. The ileum is the segment most affected by the pathogen.

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IMMUNOPHENOTYPING OF T CELL POPULATIONS IN OVINE CYSTIC ECHINOCOCCOSIS (*ECHINOCOCCUS GRANULOSUS*)

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Introduction: Ovine hydatidosis (OH; *Echinococcus granulosus*) is endemic in several European countries surrounding the Mediterranean basin. It has been reported that early, establishment-phase cysts stimulate a Th1-type immune response, while established phase cysts are associated with a Th2, immuno-suppressive type profile. The aim of the present study was to differentiate and enumerate T cell sub-populations present in the adventitia in established cysts from *E. granulosus*-naturally infected sheep.

Materials and Methods: A total of 18 established cysts were obtained from naturally infected sheep. Samples were frozen and 5 µm-thick serial sections were stained with anti-CD4, anti-CD8 and anti-Foxp3 monoclonal antibodies. Stained tissue sections were analyzed at 40× magnification. Positive cells were enumerated in five consecutive fields of the adventitial layer of the cyst. Three co-authors performed blinded, independent enumeration and mean values ± standard deviations, were calculated. Wilcoxon and Mann–Whitney tests for non-parametric distribution between CD4+ and CD8+ cells were performed and p-Values less than 0.01 were considered to be significant.

Results: In all except 5 cyst, the number of CD4+ T lymphocytes was significantly higher ($p = 0.009$) than CD8+ cells. Foxp3+ cells were present in 17 cysts and made up approximately 35% of total T cell counts in those cysts.

Conclusions: The results of the present study may contribute to identifying the immunological features involved during the different developmental stages of Cystic Echinococcosis (CE), including early establishment. Furthermore, the understanding of established-phase associated immunity may offer insights into immunotherapeutic strategies in human CE.

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CYTOKINE PRODUCTION IN STEM CELLS OF CATTLE INFECTED WITH BOVINE LEUKEMIA VIRUS (BLV)

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Introduction: Bovine leukemia virus is the etiologic agent of enzootic bovine leukosis, the most common neoplastic disease of cattle. Bone marrow is populated by hematopoietic stem cells (HSC) and by a cell population not directly involved in hematopoiesis – mesenchymal stem cells (MSC). The HSC express surface antigens CD34 and CD35 and produce and release a number of cytokines involved in the maintenance oh hematopoiesis. The aim of the study was determination the profile of cytokine production by stem cells CD34+, of cattle naturally infected with BLV.

Materials and Methods: The CD34+ cells were generated from the blood and lymphatic organs of infected with BLV and control healthy cows with the use of immunomagnetic method and monoclonal antibodies anti CD34+ (Miltenyi kit). The HSC were cultivated *in vitro* in RPMI medium supplemented with IL-4 and GM-CSF for 2 weeks. The level of cytokines: IL-6, IL-10, IL-12p40, IL-12p70, IFN-γ and TNF-α was determined in culture fluid in flow cytometer with the use of monoclonal antibodies.

Results: The levels of cytokines produced by blood HSC: IL-6, IL-12p70 and TNF-α were higher in infected cows. In bone marrow HSC of leukemic cows the greater concentrations of: both IL-12, TNF- α and IFN-γ were determined, but in spleen only IL-10 was higher in infected animals. In HSC generated from lymph node of leukemic animals only TNF- α level was lower, but all the others cytokines had greater values.

Conclusions: The infection of BLV caused statistically significant changes in cytokine expression by stem cells.

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EFFECT OF BOVINE LEUKAEMIA VIRUS (BLV) INFECTION ON SECRETIONS FROM DENDRITIC CELLS OF INFECTED CATTLE

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Introduction: Bovine leukaemia virus (BLV) belongs to *Retroviridae* family and is an etiological agent of enzootic bovine leukaemia (EBL). Dendritic cells (DCs) play crucial role in immune response, therefore investigation of their secretion profiles can provide valuable data about pathogenesis of BLV infection. The aim of the study was to analyse cytokine secretion of different DCs populations during BLV infection.

Materials and methods: Concentrations of IL-6, IL-10, IL-12(p40), IL-12(p70), IFN-γ and TNF-α were determined by ELISA and flow cytometry in cultures of myeloid DCs (mDCs), plasmacytoid DCs (pDCs) and MoDCs (monocyte derived DCs) from tissues of naturally infected cattle.

Results: DCs from blood of infected animals showed higher secretion of IL-6, IL-12(p40) and TNF-α, compared to control group. In MoDCs originated from spleen and lymph nodes of BLV-positive cattle increase in Th2 cytokines (IL-10 and IL-6) secretion and decrease in Th1 (both forms of IL-12) production was noted. In cultures of infected mDCs increase of IL-10 and decrease of IFN-γ levels was shown, which indicated the suppression of Th1 response. In BLV-infected pDCs secretion of IFN-γ was significantly higher.

Conclusions: Cytokine profiles of pDCs and blood MoDCs indicated activation of these groups during infection. Decrease in production of Th1 cytokines in favour of Th2 in case of examined mDCs, spleen MoDCs and lymph node MoDCs may suggest promotion of BLV infection development. Additionally, some of infected DCs populations through higher production of IL-6 and IL-10 may play contributory role in viral latency and oncogenesis.

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DETECTION OF THE BOVINE LEUKEMIA PROVIRUS AND ITS DISTRIBUTION IN LYMPHOID ORGANS OF NATURALLY INFECTED CATTLE AT ALEUKEMIC AND PERSISTENT LYMPHOCYTOSIS STAGES

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Introduction: Bovine leukemia virus (BLV) is the causative agent of enzootic bovine leukemia. BLV infection may remain clinically silent at the aleukemic stage (AL), cause persistent lymphocytosis (PL), or, more rarely, B cell lymphoma. The mechanisms of viral persistence and disease progression remain poorly understood at present. The aim of the study was determination of proviral load in blood and tissues of infected cows.

Materials and Methods: The study was performed on sixty one cows at AL and ten cows at PL. The distribution of the BLV provirus was evaluated by real-time fluorescence LAMP and real-time qPCR in PBMCs, bone marrow, spleen tissue, lymph node and kidney samples. Copies of proviral BLV from infected cattle were quantified by the formula: copy number BLV provirus (pol) in 1000 cells=(copy number pol)/ (copy number H3F3A/2) x1000**.**

Results: BLV provirus was detected in PBMCs and all types of investigated lymphoid tissues. In AL cattle the number of copies per 1000 cells in blood ranged from 0.03 to 7.87 copies, whereas in lymphoid organs ranged from 0.07 to 8.08 copies. In PL cattle proviral load was significantly higher than in AL animals ($p<0.05$) and ranged from 415 to 868 copies per 1000 cells in PBMCs, whereas in lymphoid organs ranged from 20.3 to 367.8 copies**.**

Conclusions: Results indicated the importance of characterizing BLV- infected cattle at AL stage during eradication programme. The risk of transmitting BLV infection from nonlymphocytotic cows may differ depending on the proviral load

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EVALUATION OF THE INTERLEUKIN (IL)-1B AND IL-8 GENE EXPRESSION IN HEALTHY SHEEP UDDERS INFUSED WITH LIVE LACTOCOCCUS LACTIS: PRELIMINARY DATA

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Introduction: Previous studies demonstrated that the intramammary infusion of live *Lactococcus lactis* into the quarters of healthy cattle leads to a rapid and considerable innate immune response. The aim of this study was to assess the effect of the intramammary infusion of live *L. lactis* on interleukin (IL)-1β and IL-8 gene expression in milk somatic cells from healthy sheep.

Materials and Methods: Eleven healthy udders were considered in the study: 7 were untreated and used as controls and 4 were infused with 2 ml of a live culture of *L. lactic* for seven consecutive days. Milk samples were collected before treatment (day 0) and 3, 7 and 15 days after the first dose (days 3, 7 and 15, respectively). Milk cell pellets were used for RNA extraction. SYBR Green RT-qPCR assays were performed by using designed sheep-specific primers for IL-1β and IL-8.

Results: IL-1β and IL-8 gene expression remained rather steady during the trial in the control group. On the contrary, fluctuations for both interleukins were observed in the treated group: a sharp drop on day 3, then a significant increase on day 7 and again a drop on day 15.

Conclusions: Our preliminary results seem to suggest that the infusion of live *L. lactis* is able to modulate the host immune response. Further investigations are needed to provide scientific evidence. This study was supported by the Italian Ministry of Health, grant number RF-2010-2373040.

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104 CANINE DISTEMPER VIRUS DETECTION IN A CHOLANGIOMA IN CDV-INFECTED FERRET

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Introduction: Canine distemper (CD) is caused by a morbillivirus of the paramyxovirus family. Canine distemper virus (CDV) infection has been demonstrated in a wide range of carnivores including Mustelidae (ferrets, minks, otters, and badgers). CD in dogs can cause serious respiratory, gastrointestinal, integumentary and nervous symptoms. In this study, we describe CDV infection in a ferret and the detection of CDV antigen in a cholangioma.

Materials and Methods: A 1-year-old ferret was submitted for evaluation. Clinical signs included dyspnea, reduced appetite, weight loss, oculonasal discharge, emaciation and mild neurological signs. After necropsy, all organs and tissue samples were fixed in 10% neutral buffered formalin andprocessed routinely with sections H&E stained. Additional sections were immunolabelled with anti-CDV monoclonal antibody.

Results: The lungs were red and hadfailed to collapse. Various sized (1~4 mm) whitish to red nodules were scattered within the liver. On cut surface of the liver, 5 mm rough textured, whitish yellow foci were observed. Histopathologically, there was mild interstitial pneumonia, cholangioma and lymphocytic cystitis, intestinal villous atrophy and lymphoid depletion of lymphoid organs. There were many intracytoplasmic and/or intranuclear inclusion bodies in the epithelial cells of the lungs, kidneys, trachea, stomach, liver, spleen, LN, Peyer's patch and urinary bladder. Immunohistochemically, positive reactions were observed in choroid plexus, adrenal glands, and in tumor cells of the cholangioma, as well as above organs with inclusion body.

Conclusions: Based on the results, this case was diagnosed as CDV infection in a ferret with cholangioma. Unusally viral antigens were detected in tumor cells of cholangioma.

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ERYSIPELOTHRIX RHUSIOPATHIAE INFECTION IN AN ALPINE IBEX (*CAPRA IBEX*)

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Introduction: *Erysipelothrix rhusiopathiae*, Gram-positive and non-spore-forming bacillus, can infect - as well as humans - a wide range of mammals, birds, reptiles, amphibians, fish and invertebrates. The pathological manifestations are generally represented by cutaneous lesions (erysipelas), endocarditis and polyarthritis. The purpose of this study is to describe a case of *Erysipelothrix rhusiopathiae* septicaemia in an Alpine ibex (*Capra ibex*).

Materials and Methods: A 12 years old male of Alpine ibex, was admitted to the Wildlife Rescue Centre in Aosta Valley Region (Northwestern Italy) and died after two days. The animal has been subjected to necropsy, followed by histological and bacteriological examination.

Results: Main gross lesions were subcutaneous and muscle sero-hemorrhagic oedema; catarrhal-hemorrhagic enteritis; fibrinous peritonitis with sero-hemorrhagic peritoneal effusion; left kidney atrophy, right kidney hypertrophy with calculosis; gallbladder with biliary stasis and thickened wall; pulmonary consolidation and fibrinous pleuritis; hydropericardium and epicardial petechial hemorrhages; prominent hepatic lobulation. The main microscopic lesion was a severe secondary systemic amyloidosis (more evident in liver, kidney and intestine), associated with a lympho-histiocytic inflammation of variable degree. *Erysipelothrix rhusiopathiae* was isolated from kidney, peritoneal effusion, pericardial fluid and brain.

Conclusions: Sporadic cases of clinical disease and mortality from *Erysipelothrix rhusiopathiae* have been reported, among wild ungulates, in wild boar (*Sus scrofa*), Iberian ibex (*Capra pyrenaica*), moose (*Alces alces*), roe deer (*Capreolus capreolus*) and white tailed deer (*Odocoileus virgianianus*). After considering the bibliographic data, this is the first report of disease caused by *Erysipelothrix rhusiopathiae* in an Italian Alpine ibex (*Capra ibex*).

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PIROPLASMOSIS IN A GREY WOLF (*CANIS LUPUS*) POPULATION IN CROATIA

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Introduction: Infection with *Babesia canis* is common in Croatian dogs suffering from haemolytic anaemia, but also in asymptomatic dogs. Captive wolves can develop babesiosis as well, while the role of free-ranging wolves in the piroplasms life cycle and impact on their health status hasn't been investigated.

Materials and Methods: Blood or different organ samples from one live-trapped wolf and 108 carcasses were screened for presence of *Babesia/Theileria* DNA. Wolf W1 was captured for movement monitoring, blood-sampled and traced for one year. Wolf W2 was euthanized after car collision. Samples from W2 were submitted for haematology, biochemistry and pathology.

Results: *Theileria* sp. closely related to *T. capreoli* was confirmed in 13.8%, while *B. canis* was found in 5.5% of animals, which presents the first report of these pathogens in free-ranging grey wolves. Haematological and biochemical findings in W1 and W2 were within reference values although merozoites were present in 0.03% and 0.09% of erythrocytes, respectively. Majority of necropsied wolves were autolytic, but no signs of hepatosplenomegaly, jaundice, anaemia, pigmenturia or DIC were found. In W2 tissue analysis revealed discrete spleen histocytosis and merozoites within erythrocytes in brain and myocardium capillaries, without other signs specific for babesiosis.

Conclusions: *B. canis* and *Theileria* sp. maintain sylvatic cycle within wolf population in Croatia. Wolves, although closely related to dogs, don't develop disease and probably serve as asymptomatic carriers. Free-ranging wolf pups most likely have developed mechanisms for piroplasm clearance like foals infected with *B. caballi* in which maternal antibodies aid in protective immunity development.

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PREVALENCE OF PORCINE CIRCOVIRUS TYPE 2 SYSTEMIC DISEASE (PCV2-SD) IN POLISH SWINE HERDS

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Introduction: Porcine circovirus type 2 systemic disease (PCV2-SD is an important syndrome limiting profitability of swine production. The aim of the study was to analyze the prevalence of PCV2-SD in Poland, in the period of 11 years.

Materials and Methods: The study was performed on lymph nodes from 340 pigs herds with clinical signs of PCV2-SD, in the years 2005 – 2015. The age of pigs varied from 4 to 20 weeks. The number of herds tested annually varied from 13 to 84. PCV2-SD was diagnosed by *in situ* hybridization (ISH) and histopathology. The results were analyzed using approximation for beta distribution for each time period to estimate PCV2-SD true-prevalence (TP) and confidence intervals (CI).

Results: The highest prevalence of PCV2-SD was found in the year 2010, reaching 14% (CI=5-28%). The rest of the results may be divided into 2 periods: first, with the higher number of PCV2-SD positive herds: from 2005 to 2009, with prevalence from 40% (CI=28%-53%) in 2009 to 54% (CI=35%-72%) in 2005 and 2007. In the second period, from 2011 to 2015, the number of PCV2-SD-positive herds varied from 31% (CI=15%-52%) in 2015 to 47% (CI=26%-69%) in 2013. These discrepancies in PCV2-SD prevalence are related to introduction of vaccines against PCV2 infections in Poland (the first one licensed in 2007), popularization of biosecurity measures and omission of newly imported pigs vaccination in the last years.

Conclusions: The results indicate that, like in other European countries, PCV2-SD still remains an actual problem in Polish swine populations.

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EXPLORING THE PATHOGENESIS OF EARLY STAGE PCV-2 INFECTION

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Introduction: Porcine Circovirus 2 causes Porcine Circovirus Associated Disease (PCVAD) which is of significant economic cost to the pig industry. The aim of this study was to use an experimental challenge model to examine the viral pathogenesis in the first two weeks post infection.

Materials and Methods: Nine three-week old piglets were infected intranasally with PCV2 and euthanased on days 3, 5, 8, 10 and 12 post challenge. Tissue and serum samples were analyzed by microscopy for PCVAD lesions, IHC for capsid protein, and qPCR for viral DNA. Results were compared to samples from non-infected age matched control animals euthanased at days 5, 8 and 12.

Results: There were no microscopic lesions suggestive of PCVAD. IHC detected viral protein in three out of nine challenged piglets. On day 5, one piglet showed labelling within the tonsil. By day 12, two piglets showed labelling in the bronchial, inguinal and mesenteric lymph nodes, the tonsil and the ileum. Viral protein was detected in the ileum of one control piglet. Piglets from both groups were viraemic at post mortem examination, and viral DNA was detected in all IHC positive tissues.

Conclusions: A variable pattern of viral protein expression was detected in the early stages of experimental PCV-2 infection. This pattern does not resemble that seen in chronic experimental infections or in PCVAD cases. The IHC results may indicate the primary site of viral replication or secondary replication following viraemic spread. It is thought that control animals were infected during the experiment.

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IMMUNOHISTOCHEMICAL DETECTION OF CLASSICAL SWINE FEVER VIRUS IN DIFFERENT FETAL TISSUES OF NATURALLY INFECTED SOWS

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Introduction: Classical Swine Fever virus (CSFV) is able to cross placenta during all stages of gestation. The outcome of trans-placental infection of fetuses mainly depends on the time of gestation. Different studies have shown that intrauterine infection during second trimester of gestation is important for developing immune-tolerance in pigs and can also lead to the birth of persistently viraemic piglets which areone of the most important sources of CSFV shedding in domestic pig population. The aim of our study was to demonstrate CSFV in different fetal tissues of naturally infected sows.

Material and methods: Tissue samples from 18 pig fetuses (70-90 days after insemination) originated from 3 naturally infected pregnant sows were collected after euthanasia. Fetal spleen, tonsils, umbilicus, brain and kidney were fixed in 10% neutral buffered formalin. After processing, 4-5µm tissue sections were stained with hematoxylin-eosin. Immunohistochemistry was performed using monoclonal antibody, WH303 (APHA Scientific, UK) for E2 glycoprotein of CSFV, and Novolink Polymer Detection Systems (Leica biosystems, Germany).

Results: Histological examination revealed lymphocyte hyperplasia in spleen and tonsilar crypts, massive hemorrhage in kidneys as well as CSFV E2 expression, predominantly in endothelial cells of kidney, brain, tonsils and umbilicus blood vessels. E2 glycoprotein was expressed in fetal tonsilar lymphocytes, renal tubulocytes and glial cells.

Conclusions: According to our knowledge, this is the first report of immunohistochemical detection of CSFV in different fetal tissues from naturally infected sows. Further investigation should clarify development of immunotolerance in fetal pigs infected with CSFV.

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SPORADIC CASES OF MULTIPLE HAEMORRHAGES IN PIGS IN GREAT BRITAIN

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Introduction: In Great Britain it is unusual for pigs to present with widespread haemorrhagic lesions. The investigation of sporadic deaths in individual pigs showing haemorrhagic disease is described.

Materials and Methods: Five pigs with multiple haemorrhages were investigated in 2011-13 under the subsidised Government (Defra) scanning surveillance programme. Four cases were in minority breeds and one in a commercial hybrid pig.Swine fevers were ruled out by the competent veterinary authority. Investigation was by gross, clinical and microscopic pathology, microbiology and toxicology.

Results: Ecchymoses and petechiae were present in multiple sites. Lymphoid tissue showed no evidence of PCV2-associated lesions. No significant bacteria were isolated. The commercial pig was infected with border disease virus (BDV). Severe megakaryocyte hypoplasia and thrombocytopenia were detected in two pigs. Anticoagulant rodenticide was detected in the livers of two others.

Conclusions: This case series describes several potential aetiologies which must be considered for haemorrhagic disease in pigs once suspicion of swine fevers has been ruled out. Ruminant pestiviruses should be considered where risk factors for infection are present; in the hybrid pig *in utero* BDV infection resulted from earlier pregnant sow contact with lambing sheep. Possible immune mediated thrombocytopenia caused the haemorrhages in two pigs. Ingestion of rodenticide may have contributed to the haemorrhagic disease in two other pigs, although concentrations were not considered sufficiently high to be the sole cause of lesions. Investigation of further cases will help determine whether there is a previously unrecognised disease, of undetermined aetiology, affecting individual pigs.

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SYSTEMIC MYCOBACTERIOSIS IN A PIG

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Introduction: Pigs are susceptible to mycobacteria of the *Mycobacterium (M.) avium complex* and *M. tuberculosis complex*. In developed countries the incidence of *Mycobacteria* in pigs is low and birds have been implicated as the source of *M. avium complex*.

Case description: In this case a 4 week-old suckling piglet was evaluated. The farm reported a high morbidity of 25% with diarrhoea and a mortality of 2% over 7 days. The symptoms the animals showed were diarrhoea and dyspnoea. Macroscopically at necropsy the animals had no contents in the large intestine and the liver showed multifocal to diffuse, round to oval, not well-circumscribed yellowish areas that stretched into the parenchyma. Histology of internal organs showed many macrophages and giant cells of the Langhans type, mostly in the liver and intestine but also in the spleen and lung. The Ziehl-Neelsen special stain showed myriad acid-fast bacilli in several organs. Bacteriological investigation for the detection of mycobacteria resulted in the isolation of *Mycobacterium avium spp hominisuis*.

Results: The macroscopical lesions in the liver resembled very extensive milk spots entirely uncommon in this age. Upon histology, a granulomatous hepatitis induced by mycobacteria was visible.

Conclusions: Infection with the *M. avium complex* is known to cause lesions in pigs, but mostly in lymphatic tissues and in the intestine. In this case the pig showed a granulomatous hepatitis, lymphadenitis, splenitis and interstitial pneumonia without necrotic areas or calcifications and *M. avium spp. hominsuis* was isolated.

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INTRAHEPATIC ICTERUS CAUSED BY PCV2 – A CASE REPORT

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Introduction: In pigs, icterus is rarely observed and can be caused by infectious or non-infectious agents. Reasons for jaundice might be dysfunction of bile secretion (posthepatic icterus), hemolytic anemia (prehepatic icterus) or damage of hepatocytes (intrahepatic icterus). Jaundice is a described symptom of Porcine Cirovirus Type 2 (PCV2)-systemic disease (SD). However there may be other causative infectious agents, e.g. *Mycoplasma suis* and *Leptospira* species.

Case description: This report presents one of three necropsied 45 - 80 kg fatteners, with an intrahepatic icterus caused by PCV2, originating from a 160-pig finishing farm in Switzerland. On this farm, 20 % of the pigs showed intradermal bleedings, wasting, a reduced general health and some pigs showed jaundice. Histopathological investigation, immunohistochemistry (IHC) against PCV2 and bacteriological investigation for *Mycoplasma suis* and *Leptospira* species were performed.

Results: The major findings in this particular animal were an impressive yellow discoloration of the carcass, mild lymphocytic periportal hepatitis with intrahepatic cholestasis, evident disruption of hepatic cord architecture and degeneration and regeneration of hepatocytes, lymphocytic and granulomatous interstitial nephritis, severe depletion of lymphoid tissues, dermatitis with necrotizing vasculitis and histiocytic interstitial pneumonia. PCV2 was detected by IHC in high amounts in the lymphoid tissues, liver and lung. *Mycoplasma suis* and *Leptospira* species were not detected.

Conclusions: Jaundice as a symptom of a PCV2-SD is rarely seen, including in Switzerland. However PCV2 is the most common causative agent and should be considered as a differential diagnosis for icterus in pigs.

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PATHOLOGICAL OUTCOMES DURING AN OUTBREAK OF ENCEPHALOMIOCARDITIS VIRUS INFECTION IN A PIG FATTENING UNIT

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Introduction: Encephalomyocarditis virus (EMCV) is a *Cardiovirus* with a worldwide distribution and a broad host range. Although rodents are considered the major virus reservoir, pigs are the domestic species most susceptible to the infection, with acute myocarditis in piglets and reproductive disorders in sows.

Materials and Methods: An outbreak characterized by sudden mortality of pigs of 30 kg/BW (80 animals died) in a fattening herd lasting one month was observed in October 2015. Sudden death was the main clinical sign. For a few pigs trembling and anorexia were also reported. Necropsy was done on a selection of dead pigs, and bacteriological, molecular (PCR for PRRSV, EMCV, PRV) and histological investigations were performed.

Results: Gross examination showed congestion, pulmonary oedema, pleural and pericardial fluid transudate. Grey-white necrotic foci were evident on myocardium and epicardium. Histology showed myocardial focal areas of necrosis, calcification and interstitial infiltrations of lymphocytes and neutrophils. In addition, hepatic haemorrhagic necrosis and pancreatic islets necrosis were observed. Only the RT-PCR for EMCV resulted positive.

Conclusions: The present work describes the pathological findings associated with an outbreak of sudden mortality in pigs. The suspect of an EMCV infection, based on clinical signs and necropsy outcomes, was confirmed by histology and RT-PCR. From the onset of the first case, several relapses were observed in the farm on January, March and April 2016. The recurrence of the infection was described in a previous work. The presence of rodents can be associated with the introduction of the virus into the herd.

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AN UNCOMMON CASE OF ERISIPELAS IN SUCKLING PIGS. CLINICAL AND PATHOLOGICAL FINDINGS

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Introduction: Swine erysipelas is caused by *Erysipelothrix rhusiopathiae*. It is present in most pig farms, both in the pigs and in the environment. The microorganism is excreted via saliva, faeces or urine. The disease is relatively uncommon in pigs under 8-12 weeks of age due to passive immunity protection. The most susceptible animals are growing pigs, non-vaccinated gilts and sows up to 4th parity.

Materials and Methods: Piglets from 10 to 25 days of age, belonging to a farrow to finish farm (180 sows unvaccinated for erysipelas), showed lethargy, fever, severe cutaneous hyperaemia and sudden death. Ten litters, with around 20-30% of the suckers were involved. The epidemics lasted for 15 days. Fifteen days before the onset of the clinical signs in piglets, some pregnant sows showed anorexia and rhomboid skin lesions. Necropsy on six death piglets was performed, followed by bacteriological and histological investigations.

Results: *Erysipelothrix rhusiopathiae* was isolated. Histologically, an active hyperaemia of the renal parenchyma and cortical and medullary multifocal haemorrhages were evident. An acute thrombo-necrotic glomerulonephritis was present; glomeruli appeared hyperaemic and several capillaries contained hyaline thrombi and rare neutrophils. The tubular epithelium showed swelling and steatosis, while tubular lumen was filled by proteinaceous material. Skin showed aspects of thrombotic microvasculitis, with arterioles leukostasis in the deep dermis. Hyperaemia and haemorrhages were observed in lungs, liver, heart, spleen and lymph nodes.

Conclusions: A rare case of erysipelas in suckling piglets was reported in an unvaccinated farrow to finish herd. Bacteriological and histological findings confirmed the diagnosis.

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CLINICOPATHOLOGICAL CHARACTERISTICS OF VIRAL INFECTION AND PHYLOGENETIC ANALYSES OF VIRAL GENOMES IN REPTILES IN KOREA

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Introduction: Twenty-four viral families have been reported in animals. Until recently, 12 of these viral families had been identified in reptiles. Parvoviral, iridoviral, herpesviral, adenoviral and paramyxoviral infections have been most frequently documented in reptiles. Although various types of viruses have been reported from reptiles worldwide, they have not yet been studied and reported in Korea. Thus, the present study was designed to provide information about the molecular epidemiology, and clinicopathological findings of viruses.

Materials and Methods: In total, 34 reptiles (10 native and 24 exotic animals) were submitted for a diagnostic investigation during the last 7 years. All organs were removed, fixed in 10% buffered formalin, routinely processed and stained with H&E for histopathology. The detection and sequencing of various viruses were performed using primer sets specific to each virus. The MEGA 6.0 program was used to construct a phylogenetic tree.

Results: Adenoviral genes were detected in a tropical girdled lizard (*Cordylus tropidosternum*) and a panther chameleon (*Furcifer pardalis*). One paramyxovirus was discovered from the lizard with adenovirus. Retroviral genes were amplified in five Burmese pythons (*Python molurus bivittatus*). Phylogenetic analyses of paramyxoviruses and retroviruses showed the highest identity to the previous isolates. Partial sequencing results from the adenoviral DNA polymerase gene in the lizard indicated that it was a novel adenovirus, distantly related to a previously known reference strain.

Conclusions: Although the infection route could not be identified, this novel adenovirus was isolated from a lizard raised in a pet shop and was coinfected with a paramyxovirus.

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MUSCULAR SARCOCYSTOSIS IN TURTLES

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Introduction: Sarcocystosis (sarcosporidiosis) is a parasitical disease caused by protozoan of the genus *sarcocystosis*. For complete maturation the parasite requires two hosts. Herbivorous and omnivorous turtles are intermediary hosts that get infected by consuming plants contaminated with feces of carnivores.

Materials and Methods: A histopathological examination of 453 turtles representing several species, i.e. *Testudo horsfieldii* (Russian tortoise), *Testudo hermanni* (Hermann's tortoise), *Testudo graeca* (Spur-thighed tortoise), *Testudo marginata* (Marginated tortoise), *Testudo kleinmanni* (Egyptian tortoise), *Geochelone denticulate* (Yellow-footed tortoise), *Malacochersus tornieri* (Pancake tortoise), *Kinixys belliana* (Bell's hinge-back tortoise), *Psammobates pardalis* (Leopard tortoise) was conducted. Samples of the hind limb muscle were collected, fixed in 10% formalin, embedded in paraffin, and stained with haematoxylin-eosin (H&E), iron hematoksylin-picric acid-acid fuchsin (van Gieson), and periodic acid–Schiff (PAS), and examined under a light microscope.

Results: The histopathological examination revealed the presence of oval, round, and longitudinal cross-sections of sarcocysts inside the fibers of 149 muscle samples. Cysts were surrounded by a thick wall divided by septae into internal chambers containing banana-shaped bradyzoites. In some cases muscles with the correct number of fibers in bundles but reduced thickness, disappearing striated and enlarged nuclei, and inflammatory cells were noticed.

Conclusions: For some time it was thought that *Sarcocystis* spp. were not very pathogenic for intermediate hosts. Now it is known that parasites at the schizont stage (parasitemia) may cause not only clinical symptoms but also death of the animal. Sarcocystosis is rarely diagnosed in turtles *in vivo* because only a few methods are available.

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SERODIAGNOSTICS IN SNAKES: ANTI-BOA IMMUNOGLOBULIN ANTIBODIES AND THEIR USE TO DETECT ANTI-REPTARENAVIRUS ANTIBODIES IN SNAKES WITH BOID INCLUSION BODY DISEASE

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Introduction: Immunoglobulins (Igs) mediate the specific recognition of foreign structures, i.e. antigens. In snakes, the adaptive immune system comprises IgY and IgM, and IgD in some species. Boid inclusion body disease (BIBD), an often fatal disease of constrictor snakes, has been linked to reptarenavirus infection, but so far, the study of the serological response towards reptarenaviruses has been hampered by the lack of reagents. We have produced horseradish peroxidase (HRP) labelled anti-boa-IgY and -IgM reagents and used them to test snakes with BIBD for anti-reptarenavirus antibodies.

Materials and Methods: Rabbits were immunized with IgY and IgM purified from *B. constrictor* serum. The produced antisera were affinity purified and HRP labelled. They were tested in immunoblotting with recombinant reptarenavirus nucleoprotein (NP) as antigen, and in an indirect immunofluorescence assay (IFA) using reptarenavirus infected Vero E6 cells as the target, then applied to serum of snakes with BIBD.

Results: The reagents were applicable in both immunoblotting and IFA. Using immunoblotting reptarenavirus specific antibodies were found in 2/8 snakes with BIBD, one of these was IgY- and IgM-positive, the second only IgY-positive. The IFA suggested that the snakes mounted an immune response not only to NP, but also to reptarenavirus glycoproteins.

Conclusions: HRP-labelled anti-boa Igs are useful for serodiagnostics in snakes. Our pilot results from boas with BIBD indicate that affected animals do not readily mount an immune response to reptarenaviruses. Whether the lack of the latter is relevant for the development of the disease is a crucial questions that requires further investigations.

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SEPTICEMIA CAUSED BY CITROBACTER BRAAKII INFECTION IN A CAPTIVE NILE CROCODILE (CROCODYLUS NILOTICUS)

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Introduction: Reports documenting *Citrobacter* infections in crocodiles involve a few cases of American alligators (*Alligator mississippiensis*). Affected animals become lethargic with reduced muscle tone, limb paralysis and cutaneous ulcerations and there may be septicemia with necrotic lesions in multiple tissues. The aim of this study was to describe pathological findings associated to *Citrobacter* infection in a crocodile.

Materials and Methods: A 24-year-old male captive Nile crocodile (*Crocodylus niloticus*) was found died unexpectedly at Bioparc Valencia (Spain). The animal was kept for over 4 years and had no previous clinical history. Two other crocodiles kept in the same enclosure are clinical healthy at the time this text is written. The animal was necropsied and tissue samples were routinely processed and stained with Ziehl-Neelsen. Additionally, samples from heart, liver and lungs were conserved for microbiological analysis.

Results: Postmortem examination revealed multiple cutaneous ulcerations located at ventral region, extremities and tail as well as high amount of sero-fibrinous exudate in pleural cavity and multiple white-yellowish multifocal nodules associated to caseous necrosis affecting lungs, endocardium, liver and spleen. Histopathologic examination of affected tissues revealed a moderate amount of well-demarcated histiocytic and heterophilic granulomas and extended areas of necrosis with numerous intralesional bacteria (bacilli) and severe bacterial embolism. Ziehl-Neelsen staining showed scant intralesional acid-fast bacteria. *Citrobacter braakii* was isolated in abundance in pure culture from endocardium, liver and lungs samples.

Conclusions: Septicemia associated to disseminated granulomatous and necrotic lesions caused by *Citrobacter braakii* infection was diagnosed based on pathological and microbiological findings.

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BOID INCLUSION BODY DISEASE AND MYCOBACTERIOSIS CO-MORBIDITY IN A *BOA CONSTRICTOR*

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Introduction: Boid Inclusion Body Disease (BIBD) is a debilitating disease of captive boid snakes, associated with reptarenavirus infection. Affected animals often die from secondary bacterial infections which are considered a consequence of immunosuppression due to the functional impairment of leukocytes that also contain the inclusion bodies (IB). Here we present the case of a *Boa constrictor* with BIBD that also suffered from mycobacteriosis.

Materials and Methods: An adult female *Boa constrictor* was sacrificed after the diagnosis of BIBD on blood smears. A full post mortem examination with histology and immunohistology for reptarenavirus was performed. A frozen liver sample was used for mycobacterial culture and identification by 16S sequencing.

Results: The post mortem examination revealed no gross findings apart from a mummified foetus. Histology identified the BIBD-typical intracytoplasmic IB in almost all cell types, and a multifocal granulomatous splenitis and hepatitis. The Ziehl-Neelsen stain revealed low numbers of acid fast bacteria within macrophages in the granulomatous infiltrates; these also exhibited IBs and were found to be reptarenavirus infected. The causative bacteria were identified as a member of the *Mycobacterium simiae* complex.

Conclusions: *M. simiae* complex are environmental, non-tuberculous mycobacteria. In humans, they generally induce lesions in patients with (infectious) co-morbidities, a scenario that might also apply to the present case, providing further evidence that BIBD is indeed immunosuppressive. Apparently, however, BIBD does not substantially affect basic leukocyte functions, such as phagocytosis. No direct cause was found for the foetal death, but general malaise in the course of mycobacteriosis must be considered.

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CYTOKINE RESPONSES OF OVINE LUNG FOLLOWING EXPOSURE TO BOVINE RESPIRATORY SYNCYTIAL VIRUS

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Introduction: This study sought to determine the immunohistochemical expression of interleukin-1 beta (IL-1β), tumor necrosis factor alpha (TNFα), interferon gamma (INFγ), IL- 4, IL-6, IL-8, IL-10 and IL-12 and to measure the levels of these cytokines in lung tissue from lambs experimentally infected with BRSV.

Materials and Methods: Lambs (*n* = 15) were inoculated at 2 days of age with 20 mL of viral inoculum ($1.26x10^6$ $TCID_{50}$ per mL) or sterile media (*n* = 15). Vital signs (rectal temperature, pulse and respiratory rates) were monitored daily in control and infected lambs. Lambs were euthanatized and necropsied at 1, 3, 5, 7 and 15 days post inoculation.

Results: Findings demonstrated a temporal association between pulmonary expression of these cytokines and lung pathology in BRSV-infected lambs. 1.- IL-4 and IL-10 are not primarily involved in the pathogenesis of BRSV infection in neonatal lambs; 2.- A significant increase of IL-1b, TNFa, INFγ and IL-6 proteins and labeled cells was found suggesting that these cytokines may play a major role in enhancing the biological response to BRSV, contributing to the development of lung lesions in BRSV-infected lambs. 3.- A significant increase of concentrations and number of immunolabeled cells of IL-8 and IL-12 was observed in infected lamb lungs throughout the study.

Conclusions: Given the marked induction of IL-8 and IL-12, anti-cytokine agents targeting these inflammatory cytokines may be useful in the prevention and treatment of BRSV, in conjunction with measures to combat the causative pathogen and prophylactic methods aimed at preventing infection.

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DIFFERENTIAL CYTOKINE PRODUCTION BY NEONATAL OVINE LUNG IN RESPONSE TO EXPERIMENTAL *MANNHEIMIA HAEMOLYTICA* INFECTION

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Introduction: The immunohistochemical expression and the lung extracts concentrations of Interleukin-1 Beta (IL-1β), Tumor Necrosis Factor Alpha (TNFα) and Interleukin-8 (IL-8) in the lung of lambs experimentally infected with *Mannheimia haemolytica (Mh)* were investigated.

Materials and Methods: The lambs were randomly assigned to 2 groups: infected and uninfected controls. The inoculum in each lamb of the infected group was 1.5×10^9 colony-forming units of the *M h* in 5 mL sterile nutrient broth. The control lambs were inoculated with 5 mL of sterile nutrient broth. The control and infected animals were killed from 1 to 15 days post-infection (dpi).

Results: These findings demonstrate a temporal association between pulmonary expression of these cytokines and lung pathology in ovine pulmonary pasteurellosis. Given that the lung expression of IL-8 was much greater than that of TNFα and IL-1β, the anti-cytokine agents directed at this mediator could be more useful in the prevention and treatment of this disease.

Conclusions: The results of this study suggest that IL-1β, TNF-a and IL-8 inflammatory cytokines may play an important role in enhancing the biological response of *Mh* and contribute to the development of the lung lesions in ovine pulmonary pasteurellosis. Our findings indicate that IL-8 is the dominant inflammatory cytokine expressed within the lungs in ovine pasteurellosis, accordingly anti-cytokine agents targeting this mediator may be most useful in the prevention and treatment of this disease, provided as a complementary measure to combat the causative pathogen agent, together with prophylactic measures to prevent the infections.

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PATHOGENESIS OF PASTEURELLA MULTOCIDA IN RABBITS. LIGHT AND ELECTRON MICROSCOPICAL STUDIES.

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Introduction: The variability in clinical signs of Pasterurellosis in rabbits may be influenced by different *P. multocida* virulence factors. The objective of the present study is to describe the pathological changes associated with a virulent strain of *P. multocida* in rabbits by light and scanning electron microscopy.

Materials and Methods: Sixteen rabbits (8-9 weeks-old) were injected with 1ml of 10^6 cfu/ ml in PBS. Two rabbits were inoculated with l ml PBS and used as a control. Samples from the nasal mucosa (both sides), middle part of trachea and from all the lobes of the lungs from both exposed and control groups were taken at 1,2,3,4,5,6,7, and 14 days post-inoculation and processed for light microscopy. The nasal mucosa at 14 days post-inoculation was processed for scanning electron microscopy.

Results: The nasal mucosal changes were characterised by erosion of the nasal mucosa and hypertrophy of mucus glands at the 4th day post- inoculation and the submucosa had focal aggregation of lymphocytes. The tracheal changes were manifested by hyperplasia and hypertrophy of mucus cells, and disorganization of cilia. The lungs showed features of fibrinous pneumonia and presence of inflammatory cells during infection. Desquamation and attachment of bacteria of the nasal epithelium were observed by Scanning Electron Microscopy (SEM).

Conclusions: Broad capsule of *P. multocida* by SEM could be attributed to its main pathogenic virulence mechanisms. Acute fibrinous pneumonia and presence of inflammatory cells were evident. Desquamation of the nasal cavity and attachment of bacteria were observed even at 14 days post-inoculation by SEM suggesting rhinitis.

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PULMONARY HYPOPLASIA AND ANASARCA SYNDROME – A NEWLY DIAGNOSED GENETIC DISORDER IN CIKA CATTLE

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Introduction: Pulmonary hypoplasia and anasarca (PHA) syndrome is a rare congenital abnormality in cattle that is characterised by hydrops fetalis including extreme anasarca and pulmonary hypoplasia/aplasia. This report describes the first two cases of PHA syndrome in Cika *cattle,* a newly diagnosed recessively inherited genetic defect in this autochthonous breed from Slovenia.

Materials and Methods: In the year 2014 necropsy of two male stillborn Cika calves from the same herd was performed. The first calf was aborted in the seventh month of pregnancy by a 13-year-old Cika cow. The second calf was delivered *by caesarean section to* a 14-year-old Cika cow that developed severe dystocia on the due date. At necropsy samples for histopathology and molecular genetic analyses were taken.

Results: Gross lesions were similar in both cases and characterized by anasarca, hydrothorax, hydropericardium, ascites, hypoplastic lungs, absence of lymph nodes, and an enlarged heart. The first calf was also athymic. The histopathology confirmed severe oedema of the subcutis and interstitium of the organs with severely dilated lymph vessels, and pulmonary hypoplasia. The pedigree analysis showed that the calves were sired by the same bull, the dams were paternal half-sisters and the second calf was the product of a dam-son mating. The genetic analysis showed mutation in *ADAMTS3* gene.

Conclusions: The morphology and gross lesions of both calves were consistent with known forms of bovine PHA syndrome. This is the first report of PHA syndrome occurring in Cika cattle, autochthonous and endangered Slovene cattle breed.

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NODULAR TROMBICULIASIS CAUSED BY *NEOSCHOENGASTIA SIMONOVICHI* IN A GROUP OF WILD RED-LEGGED PARTRIDGES (*ALECTORIS RUFA*)

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Introduction: Trombiculiasis is an infestation with the larvae of Trombiculidae (chigger mites) characterized by acute dermatitis in humans and animals. Although many species of chiggers have been isolated from domestic and wild birds, histopathological reports on skin lesions are scarce. We report for the first time trombiculiasis caused by *Neoschoengastia simonovichi* in a group of wild red-legged partridges in Western Europe.

Materials and Methods: A juvenile red-legged partridge was submitted due to the presence of severe skin lesions detected by hunters in 4 affected partridges in the same area. Complete post-mortem and histopathological examination were performed. Chigger identification was performed based on morphological traits using a compound microscope with phase contrast optics.

Results: A severe multifocal, locally extensive and nodular dermatitis centered on feather follicles and affecting the medial aspects of the legs and caudal abdomen was observed. Skin nodules possessed a central depression (crater-like) containing variable numbers of orange mites. Histologically there was a severe nodular, necrotizing and pyogranulomatous dermatitis characterized by a central necrotic area containing mite stylostomes, surrounded by severe pyogranulomatous infiltrate. Mites were identified as *Neoschoengastia simonovichi*, a species previously known from the Odessa Province of Ukraine, in Crimea, Turkmenistan, Tajikistan, and Kyrgyzstan. within

Conclusions: This report constitutes the first record of this species in Western Europe and on this host, defining *Neoschoengastia simonovichi* as an etiological agent of trombiculiasis in wild birds. This finding is epidemiologically relevant and it would be interesting to determine whether can be associated with trombiculiasis in other animals or humans.

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MULTIFOCAL PUSTULAR DERMATITIS ASSOCIATED WITH MALASSEZIA OVERGROWTH IN A FERRET

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Introduction: Malassezia yeasts are now recognized as opportunistic pathogens that play a significant role in the development of different human and animal diseases. Very few cases of Malassezia infection in ferrets has been described so far, reporting an association with otitis media and/or chronic pruritic dermatitis.

Materials and Methods: A female, 5-year-old, spayed, ferret presented with alopecia, erythema, scales and crusts on the dorsum, flank, tail, periorbital area, labial area and ears. The animal also had slightly enlarged adrenals. A main clinical differential diagnosis of adrenal disease was made and a suitable therapy was started, but without improvement. Two skin biopsies from the neck and the tail were taken.

Results: Histologically, a moderate to severe epidermal hyperplasia with diffuse ortokeratotic hyperkeratosis, spongiosis, neutrophilic exocytosis and multifocally, intraepidermic (mainly subcorneal and intracorneal) pustles were evident. Within the superficial keratin, and in the follicular ostia, there were numerous oval or peanut-shaped yeasts (Malassezia spp.) arranged in groups. In the superficial dermis, moderate interstitial inflammatory (neutrophilic) infiltrations and edema were observed. The histological diagnosis of neutrophilic pustular dermatitis, moderate, subacute, with intracorneal yeasts of genera Malassezia was made.

Conclusions: The present case describes multifocal pustular dermatitis with hyperkeratosis, rather than a merely seborrheic dermatitis, without otitis, associated with Malessezia overgrowth. A concurrent bacterial infection may have contributed to the marked pustular reaction observed. The adrenal diseases might represent the underlying primary cause of the described condition.

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ENCEPHALITOZOON CUNICULI INFECTION IN A COLLECTION OF BARBARY STRIPED GRASS MICE (*LEMNISCOMYS BARBARUS*)

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Introduction: *Encephalitozoon cuniculi* is an obligate intracellular microsporidian parasite that commonly induces latent, subclinical infections in rabbits. Clinically apparent infections have been reported in a range of species, including various rodents, carnivores, humans and birds. Here we present the first report of *E. cuniculi* infection and disease in a group of captive Barbary striped grass mice (*Lemniscomys barbarus*).

Materials and Methods: Full gross and histological examination was performed on three Barbary striped grass mice (one found dead, two sacrificed) from a zoo collection. Immunohistology and PCR was performed for *Toxoplasma gondii*, *Neospora caninum* and *E. cuniculi*, followed by sequencing of the PCR product.

Results: The first animal had suffered from severe granulomatous pneumonia with intralesional pseudocystic or individual, 1 x 2 mm, Gram-positive protozoal structures. These were also found in brain, liver, spleen and kidneys. Protozoal infection was also confirmed in both subsequently sacrificed mice, where brain, kidney and spleen, and kidney and liver were affected, respectively. Both animals exhibited predominantly mononuclear inflammatory processes in several organs. Immunohistology and PCR with subsequent sequencing (first animal) identified the protozoan parasites as *E. cuniculi*.

Conclusions: *E. cuniculi* is a zoonotic protozoan parasite, transmitted via ingestion or inhalation of infective spores shed in the urine, rarely also through open skin wounds. To our knowledge, this is the first case of encephalitozoonosis in Barbary striped mice (*Lemniscomys barbarus*). It is most likely that the animal group was infected through (indirect) contact with rabbits in the zoo, though this has so far not been confirmed.

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IDENTIFICATION OF REGIONS OF INTEREST IN VIRTUAL MICROSCOPY BY FRACTAL DIMENSION OF NUCLEAR CHROMATIN

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Introduction: Virtual microscopy is expanding rapidly based on technology progress and availability. Its impact depends critically on the effective identification of regions of interest (ROI) to be thoroughly assessed. Fractal analysis is among the tools considered for preliminary evaluation of digital histological samples.

Materials and Methods: 540 histological digital images of mammary gland samples from 20 dogs and 17 cats were used to develop Receiver Operating Characteristic (ROC) plots. The ROC models quantified the capacity of the fractal dimension of chromatin regions to classify the presence or absence of benign or malign lesions in a picture: physiologically normal tissue / adenoma / carcinoma – for dogs, and physiologically normal tissue/ adenoma / fibroadenoma / carcinoma for cats.

Results: There is no uniform pattern of variation of the fractal dimension of chromatin regions with malignity, valid for both cats and dogs. Hence, the post-test likelihood of lesion, as provided by the ROC model is not readily useful, especially for cats. An original Fractal Index for Lesion Highlighting was derived from the ROC model and it was proved to be a robust grading method for the probability of presence of malignity characters in frames from both dogs and cats, thus enabling the identification, in a wide histological sample, of ROI suited for further assessment.

Conclusions: Using the fractal dimension of chromatin regions and statistical models, a reliable quantitative parameter was derived to enable identification of ROI in virtual microscopy histological samples.

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VIRTUAL AUTOPSY (VIRTOPSY) – A RAPID TOOL TO SUPPLEMENT TRADITIONAL NECROPSY

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Introduction: Virtual necropsy through imaging technologies, is an essential tool in pathological diagnostics with numerous advantages. Rapid overview of complex pathological processes and exact localization of lesions facilitate subsequent preparation of relevant abnormalities and efficient search for projectiles. Digital documentation of changes increases the efficacy of consultations in scientifically/forensically challenging cases and reduces the frequency of cost-intensive preparation. Disadvantages include higher costs, risk of contamination and decomposition. Here we report examples of routine diagnostic cases, such as malformation, trauma, and inflammation/degeneration.

Case Reports: *Malformation:* Stillborn goat-twins (submitted for Schmallenberg virus-screening) displaying cephalothoracopagus. The exact extent of this double malformation could be demonstrated in a minimal invasive way by imaging. Internal organs showed no alterations and were negative for SBV.

Blunt force trauma: Yorkshire terrier, submitted because of suspected animal cruelty. Using CT, pneumocephalus and an impression fracture of the temporal and parietal bones and a skull base fracture could be detected. Hemorrhages could only be demonstrated via necropsy.

Inflammation/degeneration: Horse, chronic hoof infection, euthanasia due to animal protection. On sagittal section, severe purulent arthritis of the coffin joint could be detected. Only through imaging techniques and afterwards by using parasagittal sections could a partial sequestration of the coffin bone be discovered.

Conclusions: Virtopsy is a rapid tool supplementing traditional necropsy. However, they don´t represent interchangeable techniques, only a combination of both deliver valid diagnoses. To improve understanding of disease/trauma and diagnostic imaging reports by enhanced interaction of pathologists and radiologists, the use of common terminology and orientation is mandatory.

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"ONE HEALTH - ONE MEDICINE": THE ROLE OF THE PATHOLOGIST IN AN INSTITUTE DEALING WITH BOTH HUMAN AND ANIMAL HEALTH – AN EXAMPLE

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Introduction: "One Health" has become more and more prominent as an important future goal and tool. This concept recognizes that the health of humans is connected to the health of animals and the environment. It has been defined as "the collaborative effort of multiple disciplines related disciplines to attain optimal health for people".

Materials and Methods: Resulting from the start-up of the discussions dealing with the creation of one new federal research institute out of the Institute of Public Health (WIV-ISP) and the Veterinary and Agrochemical Research Centre (CODA-CERVA), this concept was put on the table. In fact, the goal was to go for "One Medicine" or one integrated medical thinking. For that, persons with multidisciplinary national and international contacts and a background of approaching syndromes with an open, unbiased view are needed. An obvious example of such persons can be found in the group of the (veterinary) pathologists.

Results: In fact, the pathologist can play a role in a large number of tasks that are needed in a One Health-One Medicine institute, namely in the syndromic surveillance, zoonotic diseases in animals, animal experiments, quality control of veterinary and human laboratories and finally in the set-up of an orientation laboratory. The different roles will be discussed

Conclusions: Pathology stays an important part of the process of understanding disease in public and animal health. Therefore it can play an important transversal role that can provide help on several levels in a One Health – One Medicine institute.

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WHY DOGS SHOULD NOT CHEW GUM

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Introduction: Xylitol (Xylit) is a sugar alcohol commonly used as a sugar substitute with anticariogenic quality in chewing gum, tooth paste and different kinds of candy. It is also purchasable as birch sugar and used for dietary reasons due to its low caloric value in baking goods and other foods. However, despite its wide use, Xylit is extremely toxic for some animals (including dogs) starting at concentrations as low as 0.1 g/kg.

Materials and Methods: A 3-year old Hovawart presented with apathy, acute vomiting, jaundice and therapy-resistant hypoglycemia. The dog died two days after first symptoms were observed. An insulinoma had been excluded by ultrasound. A xylitol-intoxication was suspected.

Results: Autopsy of the progressively autolytic dog revealed a severely friable liver and a moderate jaundice. Liver histopathology revealed a severe hepatocellular necrosis of > 95 % of the liver cells. All stains (including Azan, Fouchet, Rhodanid, Turnbull's Blue) were negative. The toxicopathological examination of blood samples via gas chromatography-mass spectrometry (GC-MS) detected xylitol.

Conclusions: The present case shows the need for awareness when it comes to species-specific sensitivities to certain substances and their use in everyday lives. When facing therapy-resistant hypoglycemia one should always consider a xylitol-intoxication as a differential to an insulinoma. Also, it proves that even if conditions are unfavorable (severe autolysis/heterolysis) it is advisable to perform a toxicologic examination.

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CANINE BRACHIAL PLEXUS NEURITIS

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Introduction: Brachial plexus neuritis (neuropathy) is rare condition in domestic animals with very few published reports in dogs, cat and cow. This entity is a rare tumor-like, chronic inflammatory, focal or multifocal, mainly demyelinating neuropathy of unknown origin, most frequently involving some nerves of the brachial plexus.
Material and methods: The surgical specimen of the musculocutaneus nerve from the brachial plexus of the 10 years old male, Bichon Frise dog which had shown right forelimb weakness, was submitted for histopathology. Tissues were fixed in 10 % formalin, embedded in paraffin, sectioned and stained with H&E, Luxol fast blue-cresyl violet and Massons's trichrome technique.

Results: Gross findings included striking thickening, hemorrhagic discoloration and fibrosis. Histopathological studies revealed extensive inflammation and axon degeneration in the musculocutaneous nerve. The changes included marked inflammation and proliferation of the epineurial and perineurial nerve sheaths. The surrounding tissue and nerve fascicles were edematous and infiltrated very extensively by lymphocytes, macrophages and plasmocytes and sustained great loss of nerve fibers. In some fascicles all component nerve fibers appeared degenerated, in others; a part of the nerve fibers population was spared. Some nerve fascicles contained necrotic foci. Segmental myelin loss was evident in the infiltrated areas. Often demyelination was associated with invading macrophages.

Conclusions: In some studies the brachial plexus neuritis has been compared to the neuritis of the cauda equina in horses and to the Guillain-Barre syndrome in humans. An immunological pathogenetic mechanism has been suspected to underlie all three forms of neuropathy.

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132 GUT MICROBIOTA MODULATION ENHANCES AMYLOID-B UPTAKE BY MACROPHAGES OF AN ALZHEIMER'S DISEASE TRIPLE TRANSGENIC MICE MODEL

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Introduction: Microglia constitutes the first line of defense against invading pathogens or other types of brain tissue injury. Apart from resident microglia, in the brain also monocyte-derived macrophages (MDM) play a phagocytic role and are implicated in the presentation of antigens to T cells. Studies have shown that MDM are able to efficiently eliminate amyloid and confer neuroprotection by secretion of growth factors. In Alzheimer's Disease, microglia plays a controversial role: on one hand activation seems to be neuroprotective at early stages of the disease, but later loses its protective effects. On these findings, the MDM arises as a key immune cell to compensate the altered functions of resident microglia. Amyloid phagocytosis by macrophages is induced by Toll-like receptor 4 (TLR4), that should be over-expressed to enhance it, and gut plays an important role in this mechanism.

Materials and Methods: We investigated whether gut microbiome modulation by administration of probiotics (SLAB51) to 3xTG-AD mice is able to activate macrophages. Two different in vitro assays regarding macrophages activity were used to assess phagocytosis and respiratory burst .

Results: Results demonstrated a higher phagocytic activity of the treated group (+40%) versus placebo and a real activation of macrophage binding capability toward generic and specific antigens (1-42 Ab). Respiratory burst was less impressive (0,150/0,103 OD), but still indicate a positive trend in terms of macrophage oxidative metabolism increase.

Conclusions: In conclusion, for what concerns peripheral macrophages we can assume that gut microbiota modulation seems able to promote the oxidative activity and phagocytosis in Alzheimer's Disease.

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NEUROPATHOLOGICAL FINDINGS IN THE BRAIN OF CATTLE WITH CLINICAL NEUROLOGICAL SYMPTOMS IN SLOVENIA SINCE THE YEAR 2000

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Introduction: The importance of passive surveillance in cattle expressing clinical neurological symptoms declined along with the beginning of the active surveillance of bovine spongiform encephalopathy (BSE), and other neuropathology has been often neglected. We examined the brains of such clinical cases, not just for BSE, but also for other lesions.

Materials and Methods: Whole brain histopathology of 378 cattle with clinical neurological symptoms of all ages was performed from the year 2000 to 2015 (347 fallen stock from farms, 31 emergency slaughtered or sick ante-mortem from abattoirs). All were examined for BSE by histopathology and from the beginning of 2001 with the rapid-post mortem tests. All were tested for rabies with immunofluorescence test (IF) and cultured for *Listeria monocytogenes.* Whole brain histopathology was made for the lesions related to these three diseases and for other neuropathology.

Results: All samples from the passive surveillance were negative for BSE, 66 (17.5%) had morphological lesions consistent with listeriosis, two (0.5%) were positive for rabies, 21 (5.6%) had lesions consistent with malignant catarrhal fever, 29 (7.7%) with other undetermined viral infections and 22 (5.8%) with other bacterial infections, 23 (6.1%) revealed cerebrocortical necrosis (polioencephalomalacia), 5 (1.3%) tumours, 31 (8.2%) metabolic or toxicity related lesions and 179 (47.3%) only non-specific lesions.

Conclusions: Neuropathology of the brains collected during passive surveillance for BSE did not reveal any positive BSE case but was important for the diagnosis of some other infectious, metabolic and neoplastic diseases in almost half of the examined animals with clinical neurological symptoms.

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CORRELATED IMAGING FINDINGS IN MENINGEAL PATHOLOGIES OF THE SPINE

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Introduction: To improve understanding of the lesion-myelon-interface and verify a presumed extradural, intradural-extramedullary or intramedullary pathology on CT/MR images correlated macro- and microscopic sections were acquired.

Materials and Methods: Four cats and three dogs with inflammatory or neoplastic disease, seen on CT (n=1) or 1.5 T MR images (n=6), were euthanized. Sections from fresh cadavers were cut and photographed in the same level and plane as best documented on CT/MR images. Tissues were fixed in formalin, decalcified with EDTA for 60 to 80 days, embedded in paraffin wax, sectioned (2-3 µm) and stained with H&E.

Results: On CT/MR images all patients had primary extramedullary disease, two had presumed intradural-extramedullary and two presumed intramedullary extension. CT/MR features of lesions´ location correlated well with macroscopic images. However, in two cases, soft tissue edema, seen on CT/MR images, was not noticed on macroscopic sections before feedback from the radiologist. Histology confirmed CT/MR classification as either inflammatory or neoplastic disease. One dog and one cat showed meningitis and one dog and one cat showed meningitis and myelitis, respectively. In two cats primary neoplasia and in one dog metastatic neoplastic disease were evident in the spine. In three cases microscopic evidence of mild meningeal infiltration could not be seen on MR images before feedback from the pathologist.

Conclusions: Compared to pathologic sections, MR imaging is superior to detect edema, however, depiction of meningeal pathologies is limited on MR images by patient size, microchip-associated susceptibility artifacts, selected imaging plane and experience of the observer.

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135 DISEASE PROGRESSION OF NECROTIZING LEUKOENCEPHALITIS IN A MALTESE DOG BY SERIAL MRI AND FINAL HISTOPATHOLOGICAL EXAMINATION

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Introduction: Necrotizing leukoencephalitis is an idiopathic inflammatory disease affecting central nervous system in dogs, but rarely reported in Maltese dogs.

Materials and Methods: A nine-month-old female Maltese dog presented with 3-week progressive neurological signs suggestive of forebrain and brainstem lesions. Serial MRI examinations over 6 months were performed during the investigation and treatment. The patient was euthanized 12 months after the initial investigation due to poor response to treatment. Histopathological examination of the brain and cranial cervical spine was performed.

Results: MRI examination revealed multiple necrotic lesions with peri-lesion edema, mass effect and/or ring enhancement affecting the cerebral white matter, and a lesion of T2W hyper-intensity and T1W iso-intensity with contrast enhancement in the brainstem. Clinical signs improved initially but later deteriorated despite the treatment. Follow-up MRI examinations revealed diminished ring-enhancing changes, peri-lesion edema and mass effect, but also demonstrated worsening of parenchymal tissue loss and cavitation of the brainstem lesion. On histopathological examination, lesions involved the entire brain. Three lesion phases were defined based on the degree of neuropil destruction and perivascular cuffing. In acute lesions, the perivascular cuffing with CD79a and MHC-II positive macrophages/histiocytes was prominent, whereas neuropils were mostly infiltrated with CD3-positive lymphocytes and MHC-II-positive macrophages/histiocytes in subacute lesions. Malacia and/or cavitation with scanty infiltrate of lymphocytes and histiocytes was noted in chronic lesions.

Conclusions: The present case, which initially presented with atypical MRI images and later exhibited varying degrees of brain lesions on histopathological examination, illustrates the disease progression of uncommon necrotizing leukoencephalitis in Maltese dogs.

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REDUCTION OF ALZHEIMER'S DISEASE BETA-AMYLOID PATHOLOGY BY MODULATING THE GUT MICROBIOTA IN A TRIPLE TRANSGENIC MOUSE MODEL

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Introduction: Gut microbiota has a proven role in modulation of some neurodegenerative diseases progression suggesting the use of probiotics in preventive or therapeutic procedures. In the present study, a novel probiotic formulation (SLAB51) was administered to a triple-transgenic mouse model of Alzheimer's disease (AD), named 3xTg-AD, and their respective wild types (WD), with the aim to investigate the potential beneficial effects on memory deficits, amyloid plaques deposition, and neuronal apoptotic index.

Materials and Methods: Mice were organized in a treated and a control group. Animals were tested for behavioural tests: *The open field* (OF), *The novel object recognition* (NOR) tests, *The passive avoidance* and *The elevated plus maze* test (EPM). Mice were sacrificed and brains collected, weighted and macroscopically evaluated. Brain samples were treated for histological evaluation, then stained to evaluate Aβ peptides deposits, using Congo red, and immunohistochemical methods.

Results: Behavioral tests revealed that SLAB51 exerted a beneficial effect on memory deficit in AD mice. Probiotic was able to counteract the typical morphological alterations of AD including the reduction in brain weight, the decline of cortical areas and the general brain damage and shrinkage. Furthermore, SLAB51 contributed to a consistent reduction in the amount of brain Aβ.

Conclusions: These data suggest the beneficial effect of SLAB51 in counteracting brain damages typical of Alzheimer's disease.

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CHITOSAN-BASED SCAFFOLD INTEGRATION IN MICE CALVARIA DEFECT MODEL: HISTOPATHOLOGIC STUDY

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Introduction: Bone grafts are used for augmenting or stimulating bone regeneration in the reconstruction of large bone defects. Autograft is the most common; however, its use can lead to an array of complications. Scaffolds (allographs) made of several biomaterials could provide the necessary support and sufficient vascularization to allow access to nutrients to support bone regeneration, therefore minimizing undesired effects. We assessed the osteogenic, osteoinductive and osteoconductive potential of a chitosan scaffold by histopathologic means in a mice calvaria defect model.

Materials and Methods: Calvaria defects were performed in 10 nude mice. A biodegradable chitosan-based scaffold was implanted in seven animals. Animals were killed at 2, 4 and 8 weeks after implantation and pathologic examination focusing the extracellular matrix, angiogenesis, cellular components, scaffold appearance and integration was performed. All procedure was approved by an Ethical committee (A 67-482-35).

Results: After two weeks, there was a predominance of macrophages on the scaffold´s interior, multifocal angiogenesis and mild connective tissue surrounding the scaffold. At four weeks, the fibroblasts predominate in all implant area, with macrophages and giant cells multifocally distributed. At 8 weeks a more mature fibrous tissue was formed on the scaffold´s interior, some mineralization focus were visualized, multifocal organized blood vessels and some giant cells persist near chitosan threads. No evidence of infection was observed.

Conclusions: The histopathologic examination demonstrates satisfactory integration of the scaffold with surrounding tissue and normal repair. These results demonstrate that chitosan can be used as scaffold-building material for bone repair.

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LOCALIZATION OF VIRAL ANTIGENS AND BRAIN ULTRASTRUCTURAL FINDINGS IN STRIPED DOLPHINS (*STENELLA COERULEOALBA*) WITH "BRAIN-ONLY" *DOLPHIN MORBILLIVIRUS* INFECTION

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Introduction: *Dolphin Morbillivirus* (DMV), a highly pathogenic agent, may cause peculiar, "brain-only" forms of infection (BOFDI) in which viral antigen/genome is found exclusively in the brain from striped dolphins (*Stenella coeruleoalba*). These BOFDIs show morphopathological and neuropathogenetic similarities with *Subacute Sclerosing Panencephalitis* and *Old Dog Encephalitis* in *Measles Virus*-infected patients and in *Canine Distemper Virus*-infected dogs, respectively.

Materials and Methods: The brains from 3 BOFDI-affected striped dolphins was investigated ultrastructurally and by double-labeling indirect immunofluorescence (IIF), in order to characterize the submicroscopic findings and the DMV-targeted neuronal and non-neuronal cell populations.

Results: Alongside nuclear (chromatin) and cytoplasmic (mitochondrial) ultrastructural changes, we were able to demonstrate viral colonization of calbindin-immunoreactive (IR) and nitric oxide synthase-IR neurons in the brain from the 3 DMV-infected dolphins under study. Furthermore, a limited astrocytic colonization was found in these 3 animals, all of which exhibiting a prominent astrogliosis/astrocytosis.

Conclusions: To our knowledge, these should be the first submicroscopic and neuropathogenetic data about BOFDI in striped dolphins. In this respect, the marked astrogliosis/astrocytosis and the reduced DMV colonization of astrocytes in the 3 dolphins under study are of interest from the comparative pathology and from the viral neuropathogenesis standpoints when compared to ODE-affected dogs, in whose brain a non-cytolytic, astrocyte-to-astrocyte infection's spread has been reported. Further studies aimed at characterizing the complex DMV-host interactions in BOFDI-affected striped dolphins are absolutely needed.

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WITHDRAWN BY THE AUTHORS

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MENINGOENCEPHALITIS CAUSED BY *MYCOPLASMA BOVIS* IN TWO DAIRY HEIFERS

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Introduction: *Mycoplasma bovis* is the predominant pathogen isolated from calves with otitis media. Meningitis is an uncommonly reported complication of chronic severe cases.

Material and Methods: Two yearling heifers presented with head tilt and ill thrift of 2 and 6 months duration, respectively. Further neurological signs (ataxia, strabismus) then developed. Both heifers were from a 300 cow dairy herd, which in addition reported 5 cases of otitis media in the latest batch of calves.

Results: On necropsy both heifers had unilateral dense clusters of caseous granulomas at the vestibular and cochlear nerve and in the surrounding dura mater/ meninges. In one heifer, the inflammation extended along the nerves into the lateral medulla and cerebellar peduncles, while in the second animal the thalamus and occipital cortex on the contralateral side were affected. *M. bovis* was cultured from the meninges and brain parenchyma and confirmed by PCR.

Conclusion: This is a novel case of meningoencephalitis caused by *M. bovis* in cattle. It confirms the chronic persistence of the pathogen in the ear and its ability to spread along cranial nerves or through the meninges.

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CEREBRAL PHAEOHYPHOMYCOSIS DUE TO *CLADOSPORIUM* SPP. IN A LION (*PANTHERA LEO*)

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Introduction: *Cladosporium* spp. are saprophytic fungi, have a worldwide distribution and are among the most common air-borne fungi. A case of cerebral phaeohyphomycosis in a Female lion (*Panthera leo*) due to *Cladosporium* spp. is described.

Materials and Methods: A female adult lion died and was necropsied. Brain, spinal cord and lungs were collected at necropsy for histopathologic analysis, fixed in 10% buffered formalin and routinely embedded in paraffin for H&E slides sections. Genomic DNA was extracted from paraffin-embedded brain tissue and submitted for PCR using panfungal primers. Subsequently, sequencing of the amplicon was performed in both directions.

Results: Grossly, in the brain, a dark-grey abscess measuring 1.8 x 2.0 cm was compressing the adjacent occipital, parietal and frontal cortex. Microscopically, the brain abscess was non-encapsulated and composed mainly by neutrophils and macrophages. Multiple brown septated, 4-6 nm in length, fungal hyphae were observed within the areas of malacia and inflammation. In the adjacent areas, gliosis, lymphocytic perivascular cuffing and infiltration of neutrophils were observed. A fragment of about 300 bp product was amplified by PCR. Analysis of the amplicon in BLAST showed a 100% similarity with other *Cladosporium* sp. sequences.

Conclusions: Histopathologic lesions and sequencing results are consistent with cerebral phaeohyphomycosis due to *Cladosporium*, and this is the first report of this disease in a wild cat. Therefore, cerebral phaeohyphomycosis should be considered a differential diagnosis of neurologic disease in wild cats.

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142 USEFULNESS OF NEUROPATHOLOGY AND PCR FOR BRAIN SAMPLING IN RABBITS WITH ENCEPHALITOZOONOSIS

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Introduction: Brain lesions caused by different infectious agents may vary, thus a correct sampling of brain tissue for the correct diagnosis of encephalitis is fundamental. Encephalitozoonosis is a disease caused by the fungus *Encephalitozoon cuniculi* and most commonly affects the brain, kidneys, liver and lungs of mammals. So far there is a lack of a precise protocol for a correct brain sampling and postmortem diagnosis of encephalitozoonosis. We aimed to describe a sampling protocol for the etiologic diagnosis of meningoencephalitis in rabbits suspected of having encephalitozoonosis.

Materials and Methods: 18 rabbits clinically suspected of encephalitozoonosis were humanely euthanatized. Brain slices at the level of the brainstem (cerebellum and pons) and diencephalon (thalamus, occipital cortex and hippocampus) were sampled during necropsy and frozen at -80°C. PCR was performed from frozen tissues. Moreover, H&E slides were obtained from transverse sections of formalin-fixed paraffin-embedded brain tissue sections for mapping brain lesions.

Results: Histopathology: Multifocal granulomatous meningoencephalitis was observed in all cases, mostly in the occipital cortex, hippocampus and thalamus. Fungal spores were not observed in H&E sections. PCR was positive for *E. cuniculi* in 10 (55.55%) cases at the level of the thalamus/hippocampus and in 7 cases (38.88%) from the brainstem.

Conclusions: Multifocal granulomatous meningoencephalitis due to *E. cuniculi* is a rare, but dangerous condition in humans and animals. We recommend that transverse sections at the level of the hippocampus should be performed for the correct PCR diagnosis of encephalitis caused by *E. cuniculi*.

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EXPERIMENTAL VACCINE AGAINST MINK ASTROVIRUS INFECTION REDUCES THE INCIDENCE OF BRAIN LESIONS

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Introduction: Astrovirus infection in mink is associated with the syndrome “greasy kits” that causes diarrhea and results in major losses, and with a “shaking mink” syndrome, where the mink experiences uncoordinated movements. Therefore, there is great interest in developing vaccines that can reduce the disease burden and the associated economic losses.

Materials and Methods: Two astrovirus vaccines based on the full-length (C1) and the truncated (C4) capsid proteins, were tested. Fifteen pregnant mink dams were either non immunized (group 1), or immunized with C4 (group 2) or C1 (group 3) and their kits were subsequently inoculated with astrovirus to evaluate the induction of immune responses, viral shedding, development of symptoms and tissue lesions. This study focuses on the histopathology. The kits were killed at 7-8 weeks of age. Samples from brain, intestines and spleen were fixed in formalin and processed by standard histological methods.

Results: At necropsy one kit had hydrocephalus. By histology, 33%, 38% and 29% of the kits in group 1-3 respectively, had one or more intestinal lesions. The lesions were mainly flattening of intestinal villie often seen together with dilated crypts containing cellular debris. In the brain 53%, 8% and 0% of the kits in group 1-3 respectively, had lesions of non-suppurative encephalitis in the grey matter, non-suppurative cuffing and/or focal non-suppurative leptomeningitis. Spleens were without lesions.

Conclusions: The immunizations and in particular the C1 vaccine reduced the incidence of histological brain lesions, and also reduced the duration and severity of clinical symptoms, and viral shedding.

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FATAL SYSTEMIC *USUTU VIRUS* INFECTION IN TWO JUVENILE GREAT GREY OWLS (*STRIX NEBULOSA*) FROM A GERMAN ZOOLOGICAL GARDEN

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Introduction: *Usutu virus* (USUV), an African arbovirus within the genus *Flavivirus*, was introduced into Italy in 1996 and afterwards spread north causing epizootics among wild and captive birds in different European countries. In Germany, USUV was initially recovered from carcasses of wild birds from the Upper Rhine Valley in 2011.

Materials and Methods: Necropsy was performed on two three months old great grey owls from Berlin Zoo, which suffered from a hyperacute fatal disease. Native tissues were screened for USUV by RT-qPCR. Formalin-fixed and paraffin-embedded tissues were investigated by HE-staining and immunohistochemistry using a polyclonal rabbit antiserum. In addition, the virus was isolated in cell culture and further characterized by molecular methods.

Results: Main finding at necropsy in both owls were multiple foci of necrosis in liver and spleen. Additionally, several other organs including brain, kidney, lung, bone marrow, heart and skeletal muscle contained necroses of different degrees. The USUV – RT-qPCR was positive in tissue pools from both birds. By immunohistochemistry, USUV antigen was detected in vascular endothelium, parenchymal and reticulohistiocytic cells, mainly associated with the necrotic foci. Molecular phylogeny revealed that the birds were infected by a new USUV strain, provisionally named USUV-Berlin, of Africa 2 lineage.

Conclusions: The reported outbreak represents the third independent USUV introduction to Germany and its northernmost occurrence in Europe. Great grey owls seem to be suitable sentinel animals for the indication of the presence of the virus in a formerly unaffected region.

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PREVALENCE OF PARANEOPLASTIC SYNDROMES IN DOGS AND CATS TREATED IN A VETERINARY HOSPITAL IN THE CITY OF SÃO PAULO (SP, BRAZIL)

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Introduction: Paraneoplastic syndromes (PNS) are disorders that arise from tumor secretion of hormones, peptides, or cytokines, or from immune cross-reactivity between tumoral and normal tissues, thus affecting several systems, most notably the endocrine, nervous, musculoskeletal, and hematologic systems. By estimating their prevalence, clinicians may anticipate actions to improve medical care in veterinary oncology.

Materials and Methods: A retrospective study was conducted, using the medical records of dogs and cats diagnosed with neoplasms by histopathological analysis in a university veterinary hospital in São Paulo (SP, Brazil) from January 2014 to December 2015, in order to estimate the prevalence of associated PNS.

Results: One-hundred and four neoplasm patients (100 dogs, 4 cats), predominantly females with mean age of six years, were studied. Thirty-six patients (34.6%) showed signs suggestive of PNS, including anemia (33.3%), pyrexia (25%), leukocytosis (19.4%), platelet aggregation (19.4%), thrombocytopenia (16.6%), weight loss (16.6%), erythrocytosis (8.3%) and gastritis (2.7%). Each patient could have simultaneously more than one type of dysfunction. Only 2 dogs, one with mesothelioma (presenting anemia and leukocytosis) and other with hemangiosarcoma (exhibiting thrombocytopenia), had been previously diagnosed with PNS by veterinarians. As some animals had more than one neoplastic type, 138 neoplasms were identified by histopathologic evaluation, 118 (85.5%) malignant and 20 (14.5%), benign. Neoplasms associated with PNS varied, although hemangiosarcomas and adenocarcinomas were the most common. The most affected sites were mammary glands (31.9%) and pelvic limbs (18.8%).

Conclusions: Data show that PNS are underdiagnosed and perhaps, if properly identified, better outcomes could be achieved with treatment.

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PREVELANCE OF FOXP3+ CELLS IN CANINE TUMOURS AND LYMPH NODES POSITIVELY CORRELATES WITH GLUCOSE TRANSPORTER 1 EXPRESSION

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Introduction: The presence of hypoxia and regulatory T cells (Tregs) in tumours are both known to be negative prognostic factors in cancer, resulting in increased malignancy, immune evasion and treatment resistance. Studies have shown an association between the two factors in cancers of humans and mice, but no previous research has shown such a correlation in canine cancers.

Materials and Methods: Samples of 57 canine tumours and 29 canine lymph nodes of various categorisations were obtained, and sequential sections were labelled by immunohistochemistry for glucose transporter 1 (Glut1) and FoxP3 as markers of hypoxia and Tregs respectively. Up to 21 regions of interest were selected on each sample in a representative pattern and given a semi-quantitative score based on its Glut1 labelling, and the number of FoxP3+ cells at each ROI was counted. A generalised estimating equation with negative binomial log link function was used to determine an association between Glut1 expression and FoxP3+ cell count.

Results: Higher Glut1 immunoreactivity was correlated with significantly higher numbers of FoxP3+ cells in both the total tumour and total lymph node sample pools. Analysis on various sub-categories of these sample pools showed this correlation was also present within samples characterised as malignant, round cell tumours, mesenchymal tumours, epithelial tumours, lymphoma, metastatic lymph nodes and reactive lymph nodes.

Conclusions: These results indicate that Glut1 expression and FoxP3+ cells are associated in a variety of neoplasms in dogs, prompting us to speculate that hypoxia may drive Treg expansion, infiltration or induction in the tumour microenvironment.

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EXPRESSION OF GALECTIN-3 IN CANINE TUMOURS

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Introduction: Galectin-3 is one of the most studied galectins and participates in several biological processes like cell proliferation, adhesion, tissue remodelling and apoptosis. Moreover, it plays a key role in cancer development and progression. The expression of this protein is a prognostic marker for some human tumours, such as melanomas and cervical carcinomas. The purpose of this study was to investigate the immunoexpression of Galectin-3 in canine neoplasms.

Materials and Methods: Fifteen canine tumours were selected for the study (osteosarcoma, melanoma, trichoblastoma, hemangioma, squamous cell carcinoma, Sertoli cell tumour, mammary comedocarcinoma, mammary solid-type carcinoma, mammary myoepithelioma, hemangiosarcoma, hemangiopericytoma, tricholemmoma, fibroma, mast cell tumour and lipoma). A primary mouse polyclonal anti-Galectin-3 antibody (A3A12, ab2785, Abcam) was applied. For negative controls, primary antibody was replaced with normal mouse IgG under the same conditions.

Results: All tumours included in this study expressed Galectin-3, with variation in intensity, percentage of positive cells, and location of the protein between tumour types. Both cytoplasmic and nuclear positivity were observed in neoplastic and tumour-associated non-neoplastic cells. It is known that Galectin-3 functions are also related to location because, despite of been synthetized in the cytoplasm, it shuttles between cytoplasm and nucleus. Furthermore, it can also be found in cell surface and biological fluids, and is expressed by fibroblasts and macrophages, corroborating our findings.

Conclusions: Canine tumours show variable immunohistochemical patterns of Galectin-3 expression. Complementary studies are needed to evaluate the importance of this protein with respect to prognosis and treatment.

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148 p16 IMMUNOSTAINING OF CANINE SQUAMOUS CELL CARCINOMAS IS NOT ASSOCIATED WITH PAPILLOMAVIRAL DNA

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Introduction: While papillomaviruses (PVs) are an established cause of human cancer, few reports have investigated so far the association between PV and canine squamous cell carcinoma (SCC). Oncogenic human PVs consistently increase the p16 tumour suppressor protein, and its immunohistochemical demonstration can be used to ascertain whether the tumour is caused by PV infection.

Materials and Methods: In the present study, archived samples of canine SCC were tested by polymerase chain reaction for the presence of PV DNA and for p16 by immunohistochemistry. The aims were to determine the potential association between PV infection and canine SCC and to assess the utility of p16 overexpression as a marker of PV carcinogenesis in dogs.

Results: A total of 52 mucocutaneous SCCs were included in the study. Nine cases (17.3%) showed moderate p16 positivity, with no association with location, tumour degree of differentiation, histotype or mitotic activity. The canPVf/FAP64 primers amplified CPV1 DNA from 3 out of 52 tumours (5.8%), one cutaneous, one oral and one tonsillar SCC. There was no association between PV presence and p16 immunostaining.

Conclusions: These results do not support a significant role of PVs in the development of canine SCC. Furthermore, PV infection was apparently not the cause of the p16 immunostaining observed in a subset of canine SCCs. A better awareness of p16 level of expression and cellular function in canine cancer could be relevant to investigate its diagnostic and prognostic role.

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CANINE DIGITAL LESIONS: RETROSPECTIVE STUDY OF 124 CASES (2010-2015)

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Introduction: Primary tumors of the digits and nail bed are relatively common in small animal practice. Due to the restrictive anatomy of digits, neoplastic and non-neoplastic lesions express similar clinical signs, and amputation is frequently performed in order to treat, diagnose and predict the clinical outcome.

Materials and Methods: The histopathology reports of confirmed digital lesions diagnosed between 2010 and 2015 were reviewed (n=124), concerning the following clinical-pathological parameters: age, breed, gender, involved limbs and digits, and type of lesion.

Results: Malignant neoplasms accounted for 75 cases (60.5%); benign neoplasms accounted for 16 cases (12.9%); non-inflammatory and non-neoplastic lesions were 10 (8.1%) and inflammatory conditions corresponded to 23 (18.5%) cases. Mixed-breed, Labrador retriever and Rottweiler were the most common breeds. 61 dogs were female (49.2%) and 63 (50.8%) were male, with a mean age of 8.4 years (S.D.3.5). The front limb (n=52, 41.9%) were more affected than the hind limb (n=32, 25.8%). Squamous cell carcinoma was the most common malignant neoplasm diagnosed (n=30, 40%), followed by melanoma (n=16, 21.3%). Histiocytoma (n=8,50%) and inverted squamous papilloma (n=5, 31.3%) were the most common benign neoplasms. In the non-inflammatory and non-neoplastic lesions group, calcinosis circumscripta (n=4, 40%) and fibroadnexal hamartoma (n=3, 30%) were the most represented. In the inflammatory group the most common lesions were folliculitis with furunculosis and deep pyogranulomas both with 6 cases (n=6, 26.1%).

Conclusions: Our findings show that the squamous cell carcinoma is the most common malignant neoplasm, followed by melanoma, which is in accordance with the literature reviewed.

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FELINE ENDOMETRIAL ADENOCARCINOMAS; STROMAL CHARACTERIZATION FOR MUSCLE INVASION IDENTIFICATION

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Introduction: Feline adenocarcinoma of the endometrium (FEA) are more common than literature describes, and can even be present in young animals. The descriptions of these lesions have increased in recent years aiming the improvement knowledge behavior as well the prognosis prediction. As the tumour stroma is an important component in cancer performance and aggression, the aim of this work was the study of intermediate filaments (vimentina and desmin) of mesenchymal cells and alpha-actin a constituent of microfilaments, associated with the smooth muscle.

Material and methods: We used 16 samples of normal uterus and 30 FEA samples belonging to the archives of Laboratory of Histology and Anatomical Pathology of UTAD. Normal samples were separated in two stages of oestrus cycle (estrogenic stage and luteal stage). Immunolabeling was evaluated based on its intensity and type of cells stained.

Results: Normal epithelial cells as well endometrial stroma in cycling uterus, were vimentin positive. The FEA stroma was more intense than normal stroma and some neoplastic epithelial cells are positive to this marker too. Desmin and α-actin had almost overlapping staining, with weak labeling of the stroma and strong muscle positivity in both normal uterus and FEA. Some FEA cells were inconsistently positive for these two markers.

Conclusions: The invasion of muscle layers is an important feature in FEA. Desmin is the best marker for identification of this invasion than α-actin, and could be important in FEA prognosis.

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IMMUNOHISTOCHEMICAL EXPRESSION OF KERATIN 8 AND 18 IN NORMAL UTERUS, CYSTIC ENDOMETRIAL HYPERPLASIA AND FELINE ENDOMETRIAL ADENOCARCINOMA

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Introduction: Keratin-18 (CK18) and CK8 are recognized epithelial markers for histopathology diagnostic. This CK forming one of the pairs of intermediate filaments that constitute the cytoskeleton of epithelial cells. CK18 is associated with the flexible support of various cytoplasmic structures, and has been associated with numerous cellular processes such as apoptosis, mitosis and signalling, and several metabolic pathways. The changes in its expression are associated with metabolic and structural modifications, and associated with aggressiveness of certain cancers. Cystic endometrial hyperplasia (CEH) of the cat may be associated with an early stage of uterine cancer, in particular with feline endometrial adenocarcinoma (FEA). The aim of this study was to evaluate the immunohistochemistry expression of CK8 and CK18 in the cat oestrous cycle and in CEH and FEA.

Material and methods: Archival material from the Laboratory of Histology and Anatomical Pathology of UTAD was evaluates and included eight uteri in estrogenic phase and 9 in the luteal stageIn addition 10 uteri with CEH and 26 with FEA were also examined.

Results: The positivity for CK-18/8 in normal endometrium is observed in almost all epithelial cells with moderate to strong intensity. There was a non-significant decrease in CK-18/8 expression in CEH, and in FEA. This loss was clearest when compared with the normal endometrium.

Conclusions: This study allowed us to conclude that the loss of expression of CK 8/18 in FEA and CEH may be associated with cell dedifferentiation in most severe lesions. Nevertheless, follow-up studies are needed to strengthen these results.

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MUTATIONAL ANALYSIS OF C-KIT AND PDGFRA IN CANINE GASTROINTESTINAL STROMAL TUMOURS (GISTS)

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Introduction: Even though relatively rare, GISTs are the most common mesenchymal tumour of the canine GI tract and are diagnosed by the immunohistochemical expression of the receptor tyrosine kinase (RTK) KIT. Activating mutations of the proto-oncogenes c-KIT and PDGFRA drive GIST oncogenesis and are used for predicting response to RTK-inhibitors in human oncology. Since the frequency and significance of these mutations are not completely known, the aim of this study was to ascertain the type and frequency of KIT and PDGFRA mutations in a series of canine GISTs.

Materials and methods: The mutational status of c-KIT (exons 8,9 and11) and PDGFRA (exons 12 and 18) genes was explored by DHPLC, genescanning and direct sequencing using RT-PCR in a series of 17 canine GISTs confirmed by CD117, PDGFRA, sm-actin and desmin immunohistochemistry.

Results: c-KIT mutations were detected in 8/17 cases (47%) and always involved exon 11 (deletion of 3-46 pb), whereas exons 8 and 9 were wild type in all cases. PDGFRA gene mutation was identified only in one case in the exon 18 (c.2522 G>A, SNP activating mutation). Even if follow-up was not available for all cases, liver metastases were documented in 5 cases, and all these cases displayed mutation in c-KIT exon-11.

Conclusions: c-KIT exon-11 mutations occur frequently in canine GISTs and seem associated with aggressive behavior. PDGFRA activating mutation are less frequent and have never been reported before in canine GISTs. Further studies are warranted to demonstrate the prognostic and predictive role of KIT or PDGFRA mutations in canine GISTs.

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WITHDRAWN BY THE AUTHORS

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CYTOMORPHOLOGICAL ASPECTS OF PLASMA CELL TUMORS IN DOGS

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Introduction: The authors are investigating a rare form of neoplasia in our country – the plasmacytoma, both in its solitary and disseminated malignant form (multiple myeloma).

Materials and methods: The study focused on 6 cases of plasma cell tumors out of a total of 245 cases of cancer in dogs between 2010 – 2015, diagnosed by cytomorphological examination. The technique involved MGG staining of smears made from the neoplastic mass obtained by fine needle aspiration or touch-imprint (after excision).

Results: We diagnosed 6 cases of plasma cell tumor: 1 multiple myeloma (MM), 3 solitary digestive plasmacytoma of which 2 were localized on the lips and one involved the colon mucosa, 1 solitary osseous plasmacytoma and 1 extramedullary cutaneous plasmacytoma.

Conclusions: From the total of 245 cancer cases 15% were represented by round cell tumors. Out of these 15% were represented by plasma cell tumors with the following distribution: 17% MM and 83% extramedullary plasmacytoma (EMP) of which 49.8% with gastrointestinal involvement, 16.6% cutaneous and 16.6% osseous. For the MM, the cytology, the clinical and paraclinical data confirmed the diagnostic; for EMP additional information was required (histopathological exam). The MM cellularity presents a higher morphological variability comparing with the EMP, ranging from well differentiated plasma cells to anaplastic cells.

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Ki67 EXPRESSION IS NOT CORRELATED WITH AREAL DENSITY OF FoxP3+ CELLS IN AFFECTED LYMPH NODES OF TREATMENT-NAÏVE CANINE DIFFUSE LARGE B CELL LYMPHOMA CASES

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Introduction: Diffuse large B cell lymphoma (DLBCL) is the most common type of canine lymphoma, for which median overall survival times are typically less than one year. Manipulation of the tumour microenvironment, of which the FoxP3+ regulatory T cell (Treg) is a principal player, represents a potentially exciting way to curb the proliferation of neoplastic cells. Our recent work identified a negative prognostic impact of Treg frequency within neoplastic lymph nodes of canine DLBCL cases. This retrospective study explored the hypothesis that Tregs promote the proliferation of neoplastic B cells within affected nodes of DLBCL cases, employing immunohistochemistry to assess both FoxP3 and Ki67 expression.

Materials and Methods: Fifty-seven biopsies of canine nodal DLBCL were selected from archives at Bridge Pathology Ltd. Tissue microarrays were constructed, immunolabelled with FoxP3 and Ki67, and digitally scanned. FoxP3+ cells were manually counted using Image J and the area labelled with Ki67 digitally measured using *Volocity* (Perkin Elmer).

Results: DLBCL cases had a lower median areal density of FoxP3+ cells than reactive lymph nodes (27 *versus* 369 cells/mm^2 (Mann- Whitney $U = 16$, $P = 0.011$)). There was no correlation between FoxP3+ cell areal density and total Ki67+ area in DLBCL cases (Spearman's rank $r = 0.058$, $P = 0.67$, 95% CI).

Conclusions: A simple relationship between Treg areal density and neoplastic cell proliferation in treatment-naïve canine DLBCL cases does not exist. Further work is required to elucidate the impact of Tregs on the proliferation of neoplastic B cells in canine DLBCL.

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CLONAL REARRANGEMENTS IN FELINE BORDELINE IDIOPATHIC INFLAMMATORY UVEITIS: PRESUMED TRANSFORMATION INTO SOLITARY INTRAOCULAR LYMPHOMA

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Introduction: Feline intraocular lymphoma is generally considered secondary to systemic lymphoma. Recently, presumed feline solitary intraocular lymphoma S-IOL has been reported and intraocular inflammation has been hypothesized to play a role in its emergence. Human primary IOL is a rare but well characterized entity, often referred to as "masquerade syndrome", as it mimics uveitis. Our aim was to investigate cases of feline idiopathic anterior uveitis refractory to therapy that histologically were considered "borderline lesions" possibly consistent with S-IOL.

Materials and Methods: Ocular globes from 5 domestic short hair cats (mean age 8.5 years, M/F ratio=1) with an original diagnosis as idiopathic inflammatory uveitis borderline (4) and feline idiopathic lympho-plasmacytic uveitis (1) were immunophenotyped using anti-CD3-, anti-CD20, and anti-CD79a antibodies. DNA from paraffin embedded tissues was extracted for clonality.

Results: Clonal TCRG was observed in 3 bordeline and in the lymphoplasmacytic uveitis. Clonal TCRG and clonal IgH rearrangement was seen in 1 borderline case. A mixed lymphocytic inflammatory infiltrate was present in all cases as evidenced by IHC (5/5) and polyclonal T cell populations (3/5).

Conclusions: Coexistence of a mixed lymphoplasmacytic infiltrate is supportive of the hypothesis that feline S-IOL are lymphomas emerging within an inflammatory process. T cell origin prevailed within this small group of S-IOL. Clonal IgH and TCRG rearrangement may reflect an emerging B cell lymphoma with a clonal anti-tumor T cell response. Alternatively, a lymphoma with cross lineage rearrangement has to be considered. Diagnosis of S-IOL is complex and needs the support of multiple diagnostic tools.

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LONG-TERM EXERCISE TRAINING AND MAMMARY TUMORS' VASCULARIZATION: THERMOGRAPHY, ULTRASONOGRAPHY AND IMMUNOHISTOCHEMISTRY

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Introduction: Breast cancer is a leading cause of death by cancer worldwide. Angiogenesis is commonly associated with an increased risk of metastasis and poor prognosis. The beneficial effects of long-term exercise training were described in several types of cancer. This work aimed to evaluate the effects of long-term exercise training on the vascularization of mammary tumors chemically-induced in a rat model.

Materials and Methods: Experiments were approved by Portuguese DGAV (no.008961). Thirty female Sprague-Dawley rats were divided into two groups: sedentary and exercised. At 7-weeks-old, all animals were intraperitoneally injected with *N*-methyl-*N*-nitrosourea (50mg/kg). Animals from exercised group were trained on a treadmill for 35 weeks, 60 min/day. Mammary tumors vascularization was evaluated by thermography, ultrasonography (Power Doppler and B Flow) and immunohistochemistry [vascular endothelial growth factor (VEGF)-A].

Results: One animal did not adapt to the exercise protocol. Four animals from each experimental group died during the experiment. An incidence of 100% was observed with a total of 28 and 23 mammary tumors in sedentary and exercised groups, respectively. Exercise training enhanced VEGF-A immunoexpression (59.91% ± 3.11 vs. 66.04% ± 4.65) and microvessels density (11.82 ± 1.09 vs. 18.35 ± 2.93) of mammary tumors. This enhanced vascularization was confirmed by thermal (higher thermal amplitude in exercised group) and ultrasound data (1.30% ± 0.29 vs. 1.47% ± 0.27 for Power Doppler, and 2.40% ± 0.52 vs. 3.68% ± 0.88 for B Flow).

Conclusions: Long-term exercise training increased VEGF-A immunoexpression leading to enhanced tumor vascularization and less numbers of malignant lesions.

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INHIBITION OF MAMMARY TUMORIGENESIS IN THE MNU-INDUCED RAT MAMMARY TUMOUR MODEL BY PROPOLIS

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Introduction: Breast cancer is one of the most frequent malignancy in female dogs worldwide. This investigation presents the protective degree of long-term day-to-day administration of propolis in the MNU-induced rat mammary tumour model.

Materials and Methods: Thirty days old juvenile female Sprague-Dawley rats were utilised. Methyl-nitroso-ureea (MNU) was utilised to induce mammary tumours, whereas the propolis was used as a chemopreventive agent. The female rats were assigned in four groups: Group 1, inoculated with 55 mg MNU/kg body weight (BW) intraperitoneally; Group 2, inoculated with MNU and received feed with propolis (15 droplets of alcoholic extract of propolis/rat/day); Group 3 inoculated with saline and received feed with propolis (15 droplets of propolis/rat/day); Group 4 inoculated with saline. The experiment continued for 290 days. A complete necropsy survey was done, followed by the histological assessment of mammary tumours.

Results: All rats of the Group 1 developed tumours compared to 7 out of 10 rats in the Group 2. The mammary tumours induction ratio was 90.24% in Group 1 and 90% in Group 2. The average mammary tumour number/rat was 3.7±2.75 in Group 1 vs 1.8±1.39 in Group 2 ($p<0.05$). A difference was noticed in the average of mammary tumours volume (16.68±33.32 in Group 1 vs 10.76±17.17 in Group 2; $p>0.05$) and mammary tumour mass compared to final BW (9.00±10.86 in Group 1 vs 7.42±11.43 in Group 2; $p>0.05$).

Conclusions: Finally, chronic daily administration of propolis in the diet of MNU-induced rat mammary tumour model induced a significant drop in the tumour occurrence comparing to the control group.

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CHARACTERIZATION OF NON-ADHERENT CELLS FROM IPC-366, A CANINE INFLAMMATORY MAMMARY CANCER CELL LINE

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Introduction: Canine inflammatory mammary cancer (IMC) is an aggressive type of cancer with poor survival and is clinically and histopathologically very similar to human inflammatory breast cancer (IBC). Recently a triple negative IMC epithelial cell line, IPC-366, has been established. The aim was to characterized IPC-366 non-adherent cells.

Materials and Methods: Cells were characterized in terms of stem cell markers expression by flow cytometry, protein production by western blot, their capacity to form tumors *in vivo* in SCID mice. The results were compared with the human IBC cell line SUM149.

Results: Our results revealed that IPC-366 and SUM149 grew in non-adherent conditions forming long-term mammospheres with a grape-like morphology. Mammosphere pellet sections revealed the presence of abundant endothelial-like cells (ELCs) and the formation of groups by tumor cells with an occasional central lumen (similar to mammary acini), interpreted as mammospheres. Both cell lines exhibited fast growth *in vivo.* Tumors were highly infiltrative and groups of elongated cells arranged in bundles were also seen. The neoplastic cells were round and large with eosinophilic cytoplasm and a large central nucleus with one or more evident nucleoli. Anisokaryosis and anisocytosis were marked. Frequently, the presence of emboli in dermis capillaries and vascular spaces lined by highly malignant neoplastic cells (vasculogenic mimicry phenomenon) were observed. Stem cell marker expressions revealed that IPC-366 and SUM149 had mesenchymal-like characteristics. Also both showed loss of expression of E-cadherin and N-cadherin and gain of expression of fibronectin.

Conclusion: IPC-366 cell line will be helpful for future interspecies comparative new therapeutic strategies against IBC/IMC.

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160 PODOPLANIN EXPRESSION IN CANCER-ASSOCIATED FIBROBLASTS (CAFs) IN MAMMARY GLAND CANCER IN DOGS

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Introduction: Mammary gland cancers are one of the most common malignant tumours in bitches. Although in veterinary medicine there are many cell markers that are useful in the diagnosis of these neoplasms, the expression of podoplanin has not been well described yet. That protein is known as a specific marker of lymphangiogenesis and is present in lymphatic endothelial cells. Moreover, an expression of podoplanin was observed in cancer-associated fibroblasts (CAFs) in invasive ductal breast carcinoma in woman. In human the presence of CAFs shows a positive correlation with negative prognostic factors.

Materials and Methods: The study was carried out on 55 samples of canine mammary gland cancer collected from bitches of various breeds and age. Tissue samples were fixed in 7% buffered formalin and embedded in paraffin blocks. The material was stained with haematoxylin and eosin (H&E) to confirm the diagnosis (according to Goldschmidt classification) and to assess the histological malignancy grade. The immunohistochemical examination of the expression of podoplanin and Ki-67 was made using mouse monoclonal antibodies (DAKO, Denmark). The results were subjected to statistical analysis.

Results: CAFs podoplanin expression was noted in 14.5 % of cases. The presence of CAFs showed a positive correlation with the dogs' age and histological malignancy grade. Moreover, in specimens with the presence of CAFs a higher proliferative potential (high expression of Ki-67) was observed.

Conclusions: The obtained results suggest a role of CAFs in the progression of mammary gland neoplasms in bitches, similarly as it occurs in ductal breast carcinoma in women.

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IMMUNOHISTOCHEMICAL EVALUATION OF P62 IN CANINE MAMMARY TUMORS

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Introduction: P62 is a ubiquitin-binding scaffold protein considered the crossroad molecule of autophagy and apoptosis. In veterinary medicine, the role of p62 in tumors is poorly understood. The aim of this study is to evaluate the immunohistochemical expression of p62 in normal mammary tissue, in adenomas and carcinomas of the dog.

Materials and Methods: The immunohistochemical analysis were performed on thirty-six mammary tumors classified according to WHO and eight normal mammary tissues. Regional lymph nodes were analyzed when present.

Results: All normal tissues exhibited a strong, homogeneous positivity. Almost all epithelial cells showed a brown granular stain in the cytoplasm while the nucleus was negative. Only 5% of myoepithelial cells were immunostained and the stroma was always negative. In all adenomas p62 immunostaining was enough intense but the percentage of epithelial positive cells was lower (65%). In malignant tumors, the immunoreaction appeared heterogeneous both between samples and within the same sample. 19 carcinomas (68%) showed small areas strongly positive close to others hardly negative, while 9 (32%) exhibited a diffuse weak stain. Metastatic cells in lymph nodes were p62 positive in 50% of cases.

Conclusions: These data could suggest a negative correlation between p62 expression and neoplastic progression. To date, as the paucity of cases examined and the complex role of p62 in autophagy and apoptosis, we believe that is not possible to consider p62 a progression marker. In the future will be interesting to compare these results with data obtained from breast cancer studies.

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CANINE MAMMARY TUMOUR WITH SMOOTH AND STRIATED MUSCLE, OSSEOUS AND CHONDROID DIFFERENTIATION: UNUSUAL MIXED TYPE OR MESENCHYMOMA?

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Introduction: Multiple mesenchymal components, usually represented by bone, cartilage, and adipose tissue, are often part of canine mammary mixed tumours. On the other hand, mesenchymomas are rare mesenchymal neoplasms demonstrating more than two mesenchymal components. The purpose of this investigation is to describe the histopathological features of a canine mammary tumour with multiple mesenchymal components.

Materials and Methods: Two nodules, respectively in the right cranial thoracic gland (M1), and in the left inguinal gland (M5) were removed from a 13-year-old crossbreed female dog. PTAH stain and immunohistochemistry using a panel of antibodies [anti-smooth muscle actin (sma), -desmin, -calponin, -P63, -CK14, -CK19, -vimentin] was applied to M5.

Results: M5 was a well demarcated, not encapsulated, expansive, moderately cellular neoplasm composed of mesenchymal well-differentiated components (cartilage, bone, smooth and striated muscle) and <5% of mammary epithelium at the periphery of the lesion. All mesenchymal areas of the tumour expressed vimentin. Striated muscle expressed desmin and calponin and smooth muscle expressed desmin, sma and calponin. PTAH demonstrated cross striation of striated muscle. The epithelial component was CK19 positive and was associated to a layer of myoepithelium expressing calponin, CK14, P63. M1 was histologically diagnosed as carcinoma-mixed type.

Conclusions: Small areas of epithelial cells confirmed the mammary origin of M5. However, the nature of the tumour remains uncertain: if an unusual benign mixed tumour with muscle differentiation, presumably arising from the myoepithelium, or a rare benign mesenchymoma infiltrating the mammary gland.

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IMMUNOHISTOCHEMICAL EXPRESSION OF TGFβ1 IN FELINE AND CANINE MAMMARY LESIONS

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Introduction: Spontaneous mammary tumours are common in female cats and dogs. The Transforming Growth Factor (TGF) beta superfamily is ubiquitously expressed in diverse tissues. TGFβ-1 isoform regulates cell proliferation, differentiation, migration, adhesion and death; some studies suggest that it functions both as a tumour suppressor and promoter. The aim of this work was to perform a comparative study of TGFβ1 expression in feline and canine non-neoplastic and neoplastic tissues, contributing to their biopathological characterization.

Materials and Methods: We analyzed 99 samples of feline and 116 samples of canine mammary tissues obtained from UTAD's Pathology Laboratory. Normal mammary tissue from animals devoid of mammary lesions was used as control. TGFβ1 immunohistochemistry was performed by the modified avidin-biotin-peroxidase complex method.

Results: In the queen, most of the histological groups showed high expression of TGFβ1 on the epithelium, but low expression in the stroma. Regarding the canine tissues, the epithelial expression of TGFβ1 was low in most samples, regardless the histological group. In the stroma, statistically significant differences were achieved, with benign neoplasia showing low expression levels, in contrast to carcinomas ($p < 0.0001$). Significant differences were observed between feline and canine malignant tissues, with most feline carcinomas exhibiting high epithelial and low stromal TGFβ1 expression and canine tissues with opposite findings.

Conclusions: This study suggests that, probably it is not the amount of TGFβ1 available in the tumour environment that promotes tumour progression, and that a crosstalk with other pathways may play an important role in TGFβ1 signaling.

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IMMUNOHISTOCHEMICAL EXPRESSION OF P-GLYCOPROTEIN AND BREAST CANCER RESISTANCE PROTEIN IN CANINE MAMMARY HYPERPLASIA, NEOPLASIA AND SUPPORTING STROMA

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Introduction: Multidrug resistance of neoplastic cells is frequently related to the expression of ABC-transporters like P-glycoprotein (PGP) and Breast Cancer Resistance Protein (BCRP). Our study aimed to determine the expression of PGP and BCRP in the components of canine mammary hyperplasias and tumours, compared it in the different histological stages and grades and describe it in the supporting stroma.

Materials and Methods: Samples included 47 lobular hyperplasias, 10 benign and 46 malignant tumours which were classified into histological subtypes, stages (0 *in situ* carcinomas, I locally invasive and II lymphovascular emboli or regional node metastasis) and grades (I, II and III according to Peña *et al*. Vet. Path., 50(1), 94-10). Immunohistochemistry with PGP (C494) and BCRP (BXP-21) antibodies was performed.

Results: PGP and BCRP were differently expressed by each mammary neoplastic component (luminal epithelial, myoepithelial and mesenchymal) and supporting stroma. Both were significantly higher in luminal cells of carcinomas vs hyperplasias (PGP P=0.0055; BCRP P=0.0352). BCRP showed a higher expression in luminal cells of simple carcinomas vs complex and mixed carcinomas (P=0.0016). Grade II and III carcinomas had a higher PGP luminal expression than grade I (P=0.0375). The positivity of stromal fibroblasts was higher in histological stage II vs I carcinomas (PGP P=0.0339; BCRP P=0.0127), and in histological grades II vs I carcinomas (PGP P=0.0343; BCRP P=0.0368).

Conclusions: Malignant and invasive tumours were more likely to express PGP and/or BCRP in luminal and stromal components, and this expression could be associated with a more aggressive and chemoresistant phenotype.

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IS IMMUNOHISTOCHEMISTRY THE BEST METHOD TO INVESTIGATE PHOSPHATIDYLINOSITOL-3 KINASE (PI3K) EXPRESSION IN CANINE MAMMARY SAMPLES?

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Introduction: In human breast cancer, Phosphatidylinositol-3 kinase (PI3K) mutation is involved in tumour growth and resistance to hormone therapy. The aim of the present study was to standardize immunohistochemistry (IHC) by anti-PI3K antibody in canine mammary samples, and to evaluate the different immunohistochemical expression in tumour progression.

Materials and Methods: To assess the anti-PI3K antibody (clone D-9) specificity a Western Blot (WB) analysis was performed in the salivary gland and in normal canine mammary sample. Samples from 3 normal mammary gland (NMG), 5 benign (B) and 13 malignant (M) tumours were available for IHC. Immunohistochemical staining was interpreted independently by two authors and the expression was evaluated by a semi-quantitative method (<50% or ≥50%) and according to a staining intensity scale from 0 to 3.

Results: WB analysis revealed two major bands of expected molecular weight (83 and 50 kDa) of PI3K isoforms. PI3K expression was ≥50% in all sample. Low (0 and 1) and high (2 and 3) staining intensity was present respectively in 1 and 2 NMG, 1 and 4 B and 2 and 11 M tumours.

Conclusions: In this investigation, the anti-PI3K antibody (clone D-9) results were specific for canine mammary tissue. However, since immunohistochemical assessment of PI3K expression showed low variation in both extension and intensity from NMG to M tumours, IHC may not be the best technique to investigate the role of PI3K in tumour progression.

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PROLIFERATIVE ROLE OF THE SDF1-CXCR4 CHEMOKINERGIC SYSTEM IN FELINE MAMMARY CARCINOMA

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Introduction: Several studies suggest the association between high expression of the CXCR4 chemokine receptor and poor prognosis in human breast cancer. The interaction of CXCR4 with its ligand SDF-1 activates multiple signaling pathways involved in tumor cell migration and proliferation, with a possible tumor-correlation. The goal of the present was to evaluate the role of SDF-1/CXCR4 system in feline mammary carcinoma (FMC) as a model of CXCR4-overexpressing human breast cancer.

Materials and Methods: Thirty-three suspect FMCs were collected. The analysis of CXCR4 expression in tissue and primary FMC cell cultures were carried out by immunohistochemistry (IHC) and immunofluorescence (IF), respectively. *In vitro* proliferative effects of SDF-1 in FMC cultures were analyzed after exposure to SDF-1 (25-100 nM) for 24h, in presence/absence of AMD3100 (CXCR4 antagonist), measuring the cell viability by MTT test.

Results: CXCR4 expression was observed in 29/31 primary FMCs and higher levels were detected in 4/6 metastases than their primary lesions. One out of 2 benign tumors was characterized by low levels, whereas normal tissues did not show any immunoreaction. Regarding *in vitro* studies, SDF-1 induced a relevant proliferative action in 5 out of 6 FMC cultures; this effect was reverted after treatment with AMD3100.

Conclusions: These results confirm, beyond the reliability of FMC as model of study for veterinary, comparative and human oncology, the involvement of the SDF-1/CXCR4 axis in tumor progression. Furthermore, it can be hypothesized that CXCR4 inhibitors could be used as anticancer strategy.

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EXPRESSION OF RICTOR AND AKT IN CANINE AND FELINE MAMMARY TUMORS

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Introduction: The PI3K-AKT pathway together with the proteins PTEN and Rictor plays a key role in the cellular metabolism, proliferation and survival and their dysregulation is involved in different human and animal tumors.

Materials and Methods: We investigated the immunohistochemical expression of the proteins AKTSer473, Rictor and PTEN in 32 samples of canine mammary carcinomas and 6 adenomas and in 29 samples of feline mammary carcinomas.

Results: In canine carcinomas, 10 out of 32 (31.2%) were at the same time AKT and Rictor positive. We found a strong negative correlation between PTEN and AKT. A positive correlation between AKT and lymphatic invasion and a negative correlation between AKT and overall survival were observed, while only a weak negative correlation between Rictor and lymphatic invasion was present. In feline carcinomas, 23 out of 29 (79.3%) were AKT positive and again all these tumours were also Rictor positive. A strong negative correlation PTEN-AKT and a strong positive correlation AKT-Rictor were demonstrated, while both AKT and Rictor were inversely correlated with overall survival.

Conclusions: Our data confirm the role of Rictor in activating AKT by phosphorylation and the role of PTEN in inhibiting this pathway. Moreover, this study confirms the crucial role of AKT, showing its potential use as pharmacological target.

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EFFECT OF THE DOWN REGULATION OF MAST CELL INFILTRATION AND KIT RECEPTOR EXPRESSION IN CANINE LOW GRADE MAST CELL TUMORS

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Introduction: KIT (CD117) is a trans membrane receptor, with tyrosine kinase activity, and its activation is critical for mast cell homeostasis and function. Mast cells (MCs) play a key role in cancer because the molecules they secrete benefit tumor growth and progression which disrupt the surrounding matrix, stimulate angiogenesis and facilitate metastases. MCs CD117 increase during the progression of many tumors types and also transformed MCs from canine mast cell tumors express this receptor.

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

Results: A significant decrease of CD117+ MCs number and KIT receptor expression was detected in the tumors of masitinib treated dogs correlating with a decrease in cell proliferation, tumor angiogenesis and local invasion. Only apoptosis was increased. The tumor progression was delayed in comparison to controls.

Conclusions: Mast cell infiltration, cell proliferation, angiogenesis and metastasis were decreased in dogs with low grade canine mast cell tumors, by down regulating KIT receptor expression.

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CANCER-ASSOCIATED FIBROBLASTS IN CANINE MAST CELL TUMOURS: CORRELATION WITH HISTOPATHOLOGICAL GRADES

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Introduction: The high incidence, mortality, and recurrence rates of mast cell tumours (MCTs) in dogs motivate research in attempt to identify biomarkers that could predict the biological behaviour of this neoplasm. In addition to cancer cells, tumour microenvironment plays an important role in cancer progression, particularly cancer-associated fibroblasts (CAFs). There is increasing histopathological and genetic evidence that CAFs proportions or signatures may refine the prognostic assessment of tumours. Cancer-associated fibroblasts have been identified based on their expression of alpha-smooth muscle actin (α-SMA).

Materials and Methods: We evaluated the immunohistochemical expression of α-SMA in intratumoral fibroblasts of 46 canine cutaneous MCTs. Positive fibroblasts were counted in 5 high-power fields, selected from areas with the highest number of labelled cells ("hot spots"). The number of CAFs for each tumour was compared with histopathological grades.

Results: Poorly-differentiated MCTs showed higher number of CAFs when compared with well-differentiated ones. Significant differences were detected between grades when Patnaik's grading system was used (P=0.0276), and also when MCTs were graded according to the 2-tier System (P=0.0105).

Conclusions: These preliminary results indicate that the presence of CAFs in MCTs may be related to malignancy and suggest an important role for these cells in the microenvironment of canine cutaneous MCTs.

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FAMILIAL MASTOCYTOSIS IN CAPTIVE FOREST FOXES (*CERDOCYON THOUS)*

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Introduction: Systemic mastocytosis is characterized by abnormal growth and accumulation of neoplastic mast cells in several organs in a variety of animal species, being more common in the dog and much less in man, cat and ox. The present study is a familial investigation in captive forest or crab-eating foxes (*Cerdocyon thous*) that presented clinical signs suggestive of mastocytosis.

Materials and Methods: Nine members (6 females and 3 males) of a captive family of forest foxes (*Cerdocyon thous)* were studied. Fine-needle aspiration biopsies were performed in 3 of them for cytopathologic examination. Immunohistochemical staining for c-Kit and Ki-67 was also performed.

Results: Cytopathologic classification showed that 2 animals had mast cell tumor grade II (low-grade) and 1 had grade I (low-grade). The main reported symptoms were diarrhea, emesis, pruritus, blemishes and skin nodules. Leukopenia, eosinophilia and increased liver enzymes were noted. In one sample, cytoplasmic labeling for c-Kit appeared focal and diffuse and moderate immunostaining for Ki-67 was also found, suggesting a worse prognosis for the case. The other samples were negative for c-Kit and Ki-67. Six animals died possibly due to the systemic mast cell degranulation. At necropsy, the skin was the most affected site, followed by lymph nodes and spleen. Hemorrhages, edema and diffuse lymphocytic infiltrates were significant microscopic findings. Three surviving animals continue to be monitored.

Conclusions: This report of familial mastocytosis in wild canids instigates future studies of comparative oncology to find genetic mutations not previously reported in this species.

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IMMUNOHISTOCHEMICAL EVALUATION OF CELL PROGRESSION MARKERS IN CANINE ORAL MELANOMA

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Introduction: Melanoma is a common malignant canine oral tumor. Recent references suggest that some members of cyclooxygenase (COX2, EP1, EP2) and integrin family may play a role in proliferation and metastases in different tumors. The aim of our investigation was to determine cell proliferation differences in oral melanomas by using a selection of these immunohistochemical markers.

Materials and Methods: Twenty four oral melanomas (12 amelanotic, 12 pigmented) were included in histopathological and immunohistochemical evaluation. The subgrouping of melanomas was based on the number of mitoses (≤3/HPF, n=11; >3/HPF, n=13). Melan A was used on amelanotic and less pigmented tumors (n=18). All samples were tested for expression of COX2, β1 integrin, EP1 and EP2 antibodies. One-way ANOVA and Pearson's chi-squared test were used for statistical analysis.

Results: A total of 89% of samples were positive for Melan A, 100% for COX2, 96% for β1 integrin, 79% for EP1 and 79% for EP2. Expression of Melan A was significantly higher in melanomas with lower mitotic index ($p<0,05$). In almost all samples with a higher mitotic index both EP1 and EP2 (12/13) were expressed. Between melanomas with lower mitotic index 4/11 expressed only EP1 and 5/11 expressed only EP2 ($p<0,05$).

Conclusions: Outcome of our investigation confirmed previous work with Melan A and COX2. The results for β1 integrin, EP1 and EP2 point out some significant differences in their expression which can play a role in aggressive biological behavior of these tumors.

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172 PLATELET DERIVED GROWTH FACTOR RECEPTORS EXPRESSION IN DOGS AFFECTED BY MALIGNANT ORAL MELANOMAS: CORRELATION WITH PROGNOSIS

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Introduction: Oral canine MM (CMM) represents the most frequent oral neoplasm occurring in dogs. It has an aggressive behavior, rapid growth and it is locally invasive and it frequently metastasizes to regional lymph nodes and distant sites. Up to 70–75% of patients in clinical stage II–III die within 1 year after surgery despite clinical treatments. Nuclear atypia, mitotic index and Ki67 index are the most significant pathologic prognostic factors. The purpose of this study was to evaluate the expression of platelet-derived growth factors receptors (PDGFR)-α and -β in stage II and III CMMs and to correlate it with clinical outcome.

Materials and Methods: PDGFRs expression was evaluated by immunohistochemistry on 48 cases of formalin-fixed CMM samples and correlated with clinical–pathological findings included Ki67 index and outcome after surgery.

Results: PDGFRs co-expression was observed in 37.5% of cases. Positivity for PDGFR-α and -β receptor was present in 54.2 and 47.9% of cases, respectively. Ki67 values >19.5% were ascertained in 66.7% of cases. Statistical analysis showed that PDGFRs co-expression and Ki67 values > 19.5% were both associated with worse prognosis.

Conclusions: The co-expression of both isoforms may suggest that these receptors can act independently by homo-dimerization as well as by heterodimerization. PDGFRs expression suggests a role in the pathogenesis and progression of CMM, and α and β co-expression appears to be associated with a worse prognosis

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LTA4H EXPRESSION IN CANINE ORAL MELANOMAS: PRELIMINARY RESULTS

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Introduction: Leukotriene A4 hydrolase (LTA4H) is an enzyme of the arachidonic acid cascade that has been investigated for its role in inflammatory related carcinogenesis and in metastatic disease in different tumors, including canine uveal melanoma**.** Canine oral melanoma is a common, most frequently malignant tumor whose biological behavior is not always predictable based on histological features. In the present study, LTA4H expression is investigated as possible marker in canine oral melanomas.

Materials and Methods: Canine oral melanomas (*n*=16) were investigated. Mitotic index (mitoses/10 HPF) and Ki-67 index (5 HPF: percentage of positively immunostained neoplastic cells/ total neoplastic) were assessed. Immunohistochemistry was performed with a mouse monoclonal antibody anti-LTA4H and semi-quantitatively scored. RT-PCR was made on a subset of melanomas (*n*=4) to evaluate LTA4H gene expression.

Results: Mitotic index ranged 0.1-7.1 (median 1.1), Ki-67 index ranged 7.9-44,4% (median 19%). In 16/16 melanomas immunohistochemical LTA4H expression was moderate to intense in more than 70% cells, with cytoplasmic (*n*=12) or nuclear localization (*n*=4). RT-PCR relative expression values ranged 0.7-8.7.

Conclusions: LTA4H immunohistochemical expression was observed in canine oral melanoma, although in this cohort of cases no evident relation with classical criteria of malignancy (i.e. mitoses, Ki67) emerged. Genetic expression showed marked differences among cases, although apparently not related with mitotic or proliferation index. Future work, on a larger number of cases, will be focused on comparing molecular and immunohistochemical expression of LTA4H with follow up data to verify if LTA4H can be proposed as a prognostic marker in canine oral melanomas.

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CYTOTOXIC EFFECT OF CATNIP AQUEOUS EXTRACT ON MURINE MELANOMA CELLS

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Introduction: The plant-derived natural products have received considerable attention in recent years due to their diverse pharmacological properties including anticancer effects. *Nepeta cataria* popularly known as catnip, cat-mint or "*erva-de-gato"* (Brazil) is used in the folk medicine as antiseptic, antimicrobial, antioxidant and for treating rheumatic disorders. However, the anticancer activity of catnip extract has not been explored so far. The present study was aimed to investigate cytotoxic effects of catnip aqueous extract on murine melanoma cells.

Materials and Methods: RAW-264.7 macrophages and B16F10 murine melanoma cells cultivated in 96 well plates were exposed or not to catnip aqueous extract in the concentrations of 50 µg/mL, 100 µg/mL or 200 µg/mL. After 24, 48 and 72 hours of culture, adherent macrophages and B16F10 melanoma cells were submitted to Crystal violet colorimetric assay to determine cell integrity.

Results: Analyses of absorbance measurements show that catnip aqueous extract at 50 µg/mL, 100 µg/mL or 200 µg/mL concentrations reduce cell viability of B16F10 melanoma cells but not macrophages after 72 hours of treatment.

Conclusions: The present work show that aqueous extract has cytotoxic effect against B16F10 melanoma cells and can be used as a potential therapeutic anticancer agent.

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GIANT CELL GLIOBLASTOMA (HIGH-GRADE ASTROCYTOMA) IN A CAT

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Introduction: Among feline brain tumors meningiomas are the most common tumor type followed by secondary brain tumors like lymphoma. Astrocytic tumors are differentiated into low-grade, medium-grade and the most malignant form, the high-grade astrocytoma (glioblastoma). Typical features of glioblastoma are vascular proliferation and necrosis.

Materials and Methods: A 12-year-old cat presented with epileptic seizures and emesis. Neurological examination revealed disorientation and reduced reflexes. Magnetic resonance imaging (MRI) was performed. After necropsy the brain was fixed, stained with HE and reticulin silver impregnation was done. Immunohistochemical studies were performed using GFAP, S100, vimentin, cytokeratin, neuro-specific enolase, neurofilament, MIB 1and ATRX.

Results: MRI examination shows a round to oval extra-axial heterogenous lesion in the cerebral parietal lobe. The lesion has a moderate mass effect on the surrounding tissue and created mild edema in the adjacent cingulated gyrus and parietal lobe. At necropsy there was a 1 x 1.2 cm-diameter, poorly demarcated mass in the left cortical hemisphere. Histopathologically, tumor cells were polymorphic, showed high cellularity and high mitotic index (MIB1), anisocytosis and anisokaryosis, necrosis, pseudopalisade formation and multinucleated giant cells. Cells were positive for vimentin, GFAP, S100 and ATRX showed a nuclear expression for ATRX. Metastases were not observed in other organs.

Conclusions: The histopathological and immunohistopathological appearance is consistent with a glioblastoma. Cerebral glioblastoma (high-grade astrocytoma) in the cat are very rare and there is limited information in literature. This is the first report of a giant cell glioblastoma in the cat.

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EVALUATION OF THE SENSITIVITY OF CHARACTERIZED CANINE GLIOBLASTOMA CELL LINES TO RADIATION THERAPY AND TO THE ONCOLYTIC EFFECT OF AN ATTENUATED MYXOMAVIRUS STRAIN

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Introduction: Combining oncolytic viruses with radiotherapy (RT) is a promising therapeutic approach to improve the poor prognosis associated with glioblastoma (GBM) in human patients. The pre-clinical evaluation of this strategy requires a translational animal model that recapitulates the major pathologic features and radiation-induced response of the tumor. As GBM-bearing dogs represent a relevant pre-clinical model, the aim of this study was to characterize 5 canine GBM cell lines, assess their response to RT and their sensitivity to the SG33 attenuated strain of myxomavirus.

Materials and Methods: Karyotypic and phenotypic characteristics of J3T, J3TBg, SDT3G, G06A and Raffray were evaluated. The cellular intrinsic radiation sensitivity has been measured using a colony formation assay with single fractions of 0, 2, 4, 6, 8 and 10 Gy. The oncolytic effect of SG33 was assessed with a MTS assay, performed 48 hours after viral infection of cell lines, at MOI 4.

Results: In addition to chromosomal abnormalities, canine GBM cell lines demonstrated an immunohistochemical profile consistent with poorly differentiated astrocytes, based on the expression of different markers of lineage differentiation. The mean inactivation dose, ranging from 4 to 5.7 Gy, indicated radioresistant cells. The MTS assay revealed a differential sensitivity to SG33, with G06A and Raffray being more resistant to the viral oncolytic effect.

Conclusions: As their human counterparts, canine GBM cell lines exhibit radioresistance. Their sensitivity to SG33 make them relevant models for future translational *in vitro* studies aiming at evaluating the synergystic cytotoxic effect of this virus combined with RT.

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CYTOLOGICAL APPROACH TO A CANINE ACANTHOMATOUS AMELOBLASTOMA

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Introduction: A 4-years old female dog was referred to the Veterinary Teaching Hospital at the University of Cordoba due to unilateral enlargement of the right maxillary area and the presence of a single, 3.0 x 1.5 cms exophytic mass. Radiographs of the skull showed focal radiolucent lesions and Computed tomography highlighted a heterogeneously enhancing destructive mass arising from the right side of the upper maxilla. A fine-needle aspirate was performed and a smear was stained with a rapid May-Grunwald Giemsa kit.

Results: Resulting smear presented elevated cellularity and prominent blood contamination. Numerous neoplastic oval to polygonal epithelial cells formed clusters, sheets and groups with a mucinous background. Cells showed a bluish cytoplasm with occasional intracytoplasmic granules, moderate anisocytosis and anisokaryosis. A concurrent population of dysplastic squamous epithelial cells with keratinized cytoplasm was observed. A modified Von Kossa staining was performed and calcium salts were demonstrated surrounding and in-between tumoral cells. The cytological exam was consistent with an acanthomatous ameloblastoma, which was confirmed histologically.

Conclusions: Squamous cell carcinoma and epithelial odontogenic neoplasias (mainly ameloblastomas) are the most common oral malignant epithelial tumors in dogs. Both types can be cytologically confused. Moreover, the common concomitant epithelial dysplasia makes the diagnosis challenging. A pre-surgery tentative diagnosis could help the surgeon in the decision, since prognosis and treatment options vary. In this case report we describe the specific cytological pattern found in acanthomatous ameloblastoma and also describe a new staining protocol that can help to differentiate both tumors.

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CONCOMITANT DIFFUSE PULMONARY CARCINOMA AND CHRONIC INTERSTITIAL PNEUMONIA IN A CAT WITH HYPERTROPHIC CARDIOMYOPATHY: UNUSUAL PATHOLOGICAL FINDINGS AND DIFFERENTIAL DIAGNOSIS

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Introduction: Bronchioloalveolar carcinoma (BAC) may occasionally occur in a diffuse form involving most or all of the lung parenchyma. This form may mimic other, more common diffuse interstitial lung diseases in both humans and animals.

Materials and Methods: A 12-year-old spayed female European shorthair cat presented with severe dyspnoea. Echocardiography revealed hypertrophic cardiomyopathy and pleural effusion. The cat died form acute decompensated heart failure. At necropsy, lungs appeared diffusely congested and firm, with presence of multifocal to coalescing, irregular grayish areas, and sparse hemorrhages. Lung tissue samples were routinely processed for histology and immunohistochemistry for pan-cytokeratin, vimentin, CAM 5.2, MAC387, CD3, Ki-67.

Results: Lung histopathology revealed a diffuse neoplastic proliferation characterized by irregular alveolar growth with multifocal micropapillary formations. Tumour cells were large, predominantly cuboidal in shape, with marked nuclear pleomorphism, prominent nucleoli, multifocal binucleated elements, high Ki-67 proliferative index and intense positivity for pan-cytokeratin and CAM 5.2. Metastases to tracheobronchial lymph nodes or other organs were not observed. Tumour growth was obscured by simultaneous lesions related to chronic congestion and interstitial pneumonia, characterized by numerous, intra-alveolar MAC387+ macrophages with multifocal erytrophagocytosis and segmental foci of bronchioloalveolar hyperplasia.

Conclusions: Histological tumour pattern characterized by neoplastic cells budding from alveolar surface was consistent with a diagnosis of diffuse BAC with unusual presence of large, pleomorphic tumour cells. Differential diagnosis included large cell carcinoma, which is usually characterized by rosettes or solid clusters of cells occupying alveolar lumen. Extensive cytokeratin immunostaining was also helpful in the differentiation from histiocytic proliferative diseases.

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FELINE PULMONARY LANGERHANS CELL HISTIOCYTOSIS WITH MULTIORGAN INVOLVEMENT

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Introduction: Pulmonary Langerhans cell histiocytosis (PLCH) is a rare disease of aged cats (10–15 years), which causes progressive respiratory failure leading to euthanasia. PLCH occurs in humans, especially among smokers and it is believed to be a reactive disorder. A neoplastic process was favored in feline PLCH based on the cytological characteristics of the LC infiltrate and the consistent extra-pulmonary lesions.

Materials and Methods: A 13-year-old female cat presented with apathy, anorexia and adipsia during the last two weeks. Some minutes before natural death, the animal presented tachypnea with marked expiratory distress and open-mouthed breathing.

Results: At the necropsy, the animal had marked jaundice. In the lungs, two 1-3 cm, white, nodular masses were detected in the caudal lobes, with diffuse micronodular pattern in the rest of the organ. Extra-pulmonary locations such as liver, pancreas, kidneys and heart, were also infiltrated by 0.3-3 cm coalescing nodular masses. The mediastinal and gastrohepatic lymph nodes were moderately enlarged. Microscopically the lesions in the lungs consisted of severe peribronchiolar histiocytic infiltrates obliterating the airways and filling the alveolar lumens. In other organs, a multifocal infiltration effaced the original parenchyma. Histiocytes were markedly pleomorphic with some mitoses (2-3/40x), multinucleated giant cells and were immunohistochemically positive for IBA-1 and E-cadherin.

Conclusions: The E-cadherin expression of the histiocytes confirms their Langerhans cell origin. PLCH is a very unusual disease of cats and has been very infrequently recorded in the veterinary literature (7 cases).

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BRAZILIAN SAVANNA FRUIT PEQUI (*CARYOCAR BRASILIENSE*) EXTRACT INHIBITS CANINE OSTEOSARCOMA CELLS GROWTH *IN VITRO*

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Introduction: Osteosarcoma is a mesenchymal high mortality tumor of dogs and men. Cytotoxicity assays employing Brazilian exuberant rain forest flora have been widely performed to identify possible active compounds to inhibit neoplastic cells proliferation. Nevertheless, very little has been investigated about the pharmacological properties of the fruits from Cerrado, center-west Brazil's diversity-rich, predominant savanna-type vegetation. The aim of this study was to verify the cytotoxic activity of the ethanoic extract of the pequi fruit mesocarp (EECP) on osteosarcoma cells.

Materials and Methods: Established-cultured osteosarcoma cells were obtained from a cell line bank, sub-cultured and subjected to treatment with EECP in three different dosages (0,1µl, 1µl and 10µl, added to culture media) for three different periods of exposure (24, 48 and 72 hours).

Results: The 10µl, 72-hour group inhibited growth in a most conspicuous manner (28.20% growth as compared to controls, i.e., a 71.80% inhibition). However, similar results ($p>0.05$) were delivered by the the 1µl, 72 hour-group (34.22%, i.e., a 65.78% inhibition), demonstrating that the latter was more efficient since the EECP dosage was 10 times lower. The smallest IC_{50} average was 155.2 µg/mL, also in the 10µl, 72 hour-group. As the survival rate was calculated, it was presumed that, following treatment, growth was smaller in the 10µl, 72 hour-group.

Conclusions: The ethanolic extract of the pequi fruit mesocarp has a cytotoxic effect on canine osteosarcoma cells, reducing cell viability in both exposing time and concentration-dependent manner of the extract used.

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IN VITRO EFFECT OF THE INTERACTION BETWEEN VINCRISTINE AND CARPROFEN OR KETOPROFEN ON THE CANINE OSTEOSARCOMA CELL LINE (D-17)

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Introduction: Vincristine is a cytostatic drug, commonly used in treatment of various types of neoplasms in dogs and humans but not in osteosarcoma. NSAIDs are often used in veterinary because of their analgesic, anti-inflammatory and antipyretic activity. The aim of the study is to assess the viability of canine osteosarcoma cell line treated with combinations of vincristine and carprofen or ketoprofen.

Materials and methods: For tests there were chosen following concentrations: for vincristine(vin) was 0.001µg/ml, for carprofen(car) and ketoprofen(keto) was 10, 1, 0.1µg/ml. The cells were exposed to the tested compounds and their combinations for a period of 72 hours in an incubator with the constant flow of 5% CO_2 and the temperature of 37°C. The viability of the cells treated with the tested substances was evaluated using MTT assay. Four independent repetitions were performed and the results are given as the average of these values.

Results: Cell viability treated by vincristine in concentration of 0.001µg/ml, carprofen and ketoprofen in 10, 1, 0.1µg/ml was respectively, 40.94±3.8%, 97,48±0.75%, 127.68±1.1%, 133.47±2.28% and 109.31±7.02%, 130.62±3.85%, 139.28±4.53%. Treated by combination of 0.001µg/ml vincristine with 10, 1, 0.1µg/ml of carprofen was 95.08±4.59%, 101.09±2.31%, 101.84±2.75% and with 1, 0.1µg/ml of ketoprofen was 88.09±4.79%, 87.55±4.11% and 84.59±1.83%.

Conclusions: Vincristine in the presence of tested concentrations of carprofen and ketoprofen have had reduced cytotoxic activity against canine osteosarcoma. Probably, there is antagonistic interaction between tested drugs. It's better not to use a vincristine and carprofen or ketoprofen simultaneously, because they may affect adversely on efficacy of anticancer therapy.

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IN VITRO STUDY OF THE INTERACTION BETWEEN DOXORUBICIN, MELOXICAM AND SODIUM RISEDRONATE ON THE CANINE OSTEOSARCOMA CELL LINE (D-17)

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Introduction: Doxorubicin is one of the most often used cytostatic drugs in the treatment of canine osteosarcoma. Meloxicam reduces inflammation and pain symptoms which sometimes accompanying neoplastic diseases. Risedronate is often used in the treatment of bone diseases include Paget's disease or post-menopausal osteoporosis. The aim of the study is to assess the viability of canine osteosarcoma cell lines treated with combinations of doxorubicin, meloxicam and risedronate.

Materials and methods: For tests there were chosen following concentrations: for doxorubicin(-doxo) it was 0.01µg/ml, for meloxicam(melo) of 100 and 10µg/ml and for risedronate(rise) of 100µg/ml. The viability of the cells treated with the tested substances was evaluated using MTT assay. Four independent repetitions were performed and the results are given as the average of these values.

Results: Cell viability treated by doxorubicin in concentration of 0,01µg/ml, meloxicam in 100 and 10µg/ml and risedronate in 100µg/ml was respectively 64.32±4.1%, 83.4±3.36%, 125.55±1.29%, 53.6±1.46%. Treated by combination of 0.01µg/ml doxo+100µg/ml melo, 0.01µg/ml doxo+10µg/ml melo, 0.01µg/ml doxo+100µg/ml rise, 100µg/ml rise+100µg/ml melo and 100µg/ml rise+10µg/ml melo was respectively 46.08±2.66%, 63.99±3.58%, 15.51±3.43%, 10.01±3.13% and 41.81±5.58%. Treated by combination of 0.01doxo+100melo+100 rise and 0.01doxo+10melo+100 rise was 23.5±3.18% and 23.76±4,57%.

Conclusions: The presence of risedronate in combination with doxorubicin or meloxicam increased their cytotoxicity against canine osteosarcoma cells . Also, its presence in combination with doxorubicin and meloxicam increased their antiproliferative activity, but weaker than in combinations with doxorubicin or meloxicam alone. Presence of synergy between tested compounds considered that, risedronate should be used more often in treatment of canine osteosarcoma.

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SPINDLE CELL LIPOMA IN THE DOG: HISTOLOGICAL DIAGNOSTIC FEATURES

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Introduction: Canine spindle cell lipoma (SCL) is an uncommon benign spindle cell tumour that might be misdiagnosed as soft tissue sarcoma (STS) because of the high cellularity and paucity of mature adipose tissue. The aim of this report is to define typical histopathological features of canine SCL.

Materials and Methods: Dog signalment, tumour size and site were retrieved from medical records. Hematoxylin and eosin, Masson's trichrome, Alcian blue, PAS and immunohistochemistry for vimentin, smooth muscle actin (sma), S100, factor VIII-ra and mdm2 were performed. Mitotic count (MC) and MIB-1 based labeling index (LI) were assessed.

Results: Five canine subcutaneous tumors were retrieved. Tumours contained bland spindle cells with scarce eosinophilic cytoplasm, oval nucleus and inconspicuous nucleolus, intermixed with ropey collagen and Alcianophilic myxoid matrix. Three cases contained scattered mature adipocytes (less than 10%). MC was <1 in 4 cases and 1 in 1 case. LI range was 0.9-3.4. All cases expressed vimentin and were S100, sma, factor VIII-ra and mdm2 negative, ruling out peripheral nerve, muscular/myofibroblastic and endothelial cell origin. The low proliferation rate, the absence of lipoblasts and the lack of mdm2 expression ruled out fibrosarcoma, myxoid liposarcoma and dedifferentiated liposarcoma respectively. A fibroma was ruled out based on the high cellularity.

Conclusions: Histopathological features identified in this caseload (bland spindle cells, rare adipocytes, ropey collagen and myxoid matrix) paralleled descriptions of SCL in man. The identification of these morphological hallmarks is necessary to diagnose SCL and to avoid a diagnosis of STS characterized by a poorer prognosis.

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EMBRYONAL RHABDOMYOSARCOMA OF THE ESOPHAGUS IN A DOG

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Introduction: A 15 month old Great Dane presented with anorexia, lethargy and weight loss and hyperthermia for about 2 months. Recently he developed generalized stiffness and diffusely swollen distal limbs. Radiographs of the limbs showed lesions corresponding to hypertrophic osteopathy. Thoracic radiographs revealed a soft tissue opacity in the caudal mediastinum, mainly at the left side. Using contrast radiography, the caudal part of the thoracic esophagus showed dorsal displacement. Sternotomy revealed an esophageal mass in the caudal mediastinum and small biopsies were taken.

Materials and Methods: FFPE tissue biopsies were stained with H&E and IHC was performed using different antibodies.

Results: Microscopic examination showed a non-encapsulated, cellular neoplasia containing 2 cell populations. One consisted of sheets of polygonal cells with indistinct borders and eosinophilic cytoplasm with oval central nuclei, stippled chromatin and 1 basophilic nucleolus. Intermingled and arranged in streams were spindloid cells with eosinophilic cytoplasm and occasionally cross-striations and the same nuclear features. There were 2 to 5 mitoses/HPF and moderate anisocytosis and anisokaryosis. Both populations contained bi- and trinucleated cells, some of them with nuclear rowing. IHC was positive for vimentin, desmin and sarcomeric actin. Based on these results, the diagnosis of embryonal rhabdomyosarcoma was made.

Conclusions: Rhabdomyosarcomas are rare neoplasms in dogs that arise from striated muscle. Embryonal rhabdomyosarcomas occur in 23% of canine cases and are most commonly reported in the head and neck region. To the best of our knowledge, this is the first description of an esophageal embryonal rhabdomyosarcoma in a dog.

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REACTIVE ANGIOMATOSIS IN A DOG

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Introduction: Angiomatosis is a vascular endothelial proliferative disorder causing dysplasia and/or hyperplasia of blood vessels and includes a wide range of vascular lesions such as malformations, reactive vascular proliferations and neoplasms.

Materials and Methods: A 1-year-old mixed-breed neutered male dog presented with severe recurrent non-responsive external otitis with ulceration and a red-purple hemorrhagic plaque on the left pinna. A tecalbo was performed. Two months later, the dog showed similar ipsilateral head and tongue lesions. All the lesions were histologically examined.

Results: Histology of all surgical samples showed a subepithelial proliferation of variably sized vascular channels lined by well differentiated to slightly plump endothelial cells. Several vessels were enclosed and occasional clusters of anastomosing sinusoidal spaces filled by blood cells were present. The interstitium was infiltrated by neutrophils, macrophages, few plasma cells and mast cells. Severe neutrophilic ulceration was also present.

Conclusions: Based on the presence of vascular proliferation, limited nuclear atypia, inflammatory infiltration and clinical history a diagnosis of reactive angiomatosis (RA) was made. In humans the common characteristic of RA is the tendency to develop either a vasculopathic process or an inflammatory vascular reaction that generates a localized hypoxic stimulus causing the neovascularization. Human RA is often idiopathic, but can be associated with *Bartonella* sp. (bacillary angiomatosis) especially in immunocompromised patients. In this case, considering the history of external otitis, a triggering bacterial etiology could be hypothesized. Prospective investigation of further cases could provide valuable information about etiology and prognosis of RA in the dog.

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METASTATIC LIPOSARCOMA IN A DOG

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Introduction: Liposarcoma (LS) is uncommon in dogs but is the most frequent malignant mesenchymal tumour in humans. Biologic behaviour of LS in humans is partially dependent upon the subtype; dedifferentiated LSs have a poor prognosis.

Materials and Methods: A 10 year-old mixed-breed dog was examined for a subcutaneous gluteus mass. Fine-needle cytology was performed. X-ray and ultrasound did not reveal any distant involvement. The removed mass was sent for histology and immunohistochemistry (IHC). Ten months later ultrasound and CT follow-up revealed two others lesions involving the right kidney and the gastric wall. Both lesions were surgically removed and histologically examined. No adjuvant chemotherapy was performed.

Results: Cytology revealed a spindle cell undifferentiated neoplasia. Histopathology of the gluteus and kidney masses revealed a neoplastic proliferation of spindle/round pleomorphic cells characterised by vacuolated cytoplasm and high mitotic index. Frequent multinucleated cells, rare lipoblasts, adipose differentiation and S100 immunoreactivity were features consistent with a poorly differentiated LS. The gastric mass was a leiomyoma. The dog is still alive 14 months after the first surgery.

Conclusions: In humans, retroperitoneal sarcomas are rare and display a vast array of histological subtypes, among which LS are the most common. No cases of retroperitoneal LS are reported in veterinary medicine. Diagnosis of poorly differentiated LSs is not straightforward and IHC is mandatory for the correct classification. Few data about the biological behavior and prognosis of LS in dogs are available. This report adds further information about LS biology in dogs.

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ATYPICAL VASCULAR TUMOR IN A FEMALE *NYMPHICUS HOLLANDICUS*

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Introduction: Vascular tumors are infrequently described in the exotic bird pathology. Histopathologic diagnosis includes well or less differentiated pleomorphic, endothelial cells and immunohistochemistry results are varied making relevance difficult.

Materials and Methods: An eight year old female *Nymphicus hollandicus* presented at the Faculty of Veterinary Medicine, after severe bleeding of a left cheek nodule. The bird died shortly and was submitted for imaging and pathological investigations which inlcuded radiology, gross, histological cytopathological and immunohistochemical examinations.

Results: Radiologic examination revealed a compact tissue deriving from the left cheek with no bone infiltration. On gross examination, the bird was in poor body condition and anemic. The facial nodule was reddish-black, soft, hemorrhagic and necrotic. Internal organ had several other reddish-black nodules on the left myocardium, lung, ventriculus and ovary. Cytology revealed blood and inflammatory cells. Histopathology of the facial nodule showed mainly massive blood-filled areas associated with inflammatory cells, necrosis, fibrin and dermic clumps of polygonal and fusiform neoplastic cells. The lung revealed groups of pleomorphic, malignant cells, either compact or with tendancy of neoformation of blood vessels. Immunohistochemistry for Vimentin and von Willebrand Factor antibodies was inconclusive in this case. In addition, a subcutaneous, yellowish mass was observed in the coelomic region and histopathologic examination revealed a lipoma.

Conclusions: This is the first case report of an atypical malignant vascular tumor with multicentric or metastatic evolution and concurrent evolution of a lipoma on the female of *Nymphicus hollandicus* in Romania.

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RECURRENT MALIGNANT PERIPHERAL NERVE SHEATH TUMOR IN THE AFRICAN HEDGEHOG (*Atelerix albiventris*): HISTOLOGIC AND IMMUNOHISTOCHEMICAL FINDINGS

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Introduction: Soft tissue sarcomas are a heterogeneous population of mesenchymal tumors arising preferentially in middle to older aged animals. Malignant peripheral nerve sheath tumors (MPNSTs) arise from Schwann cells, perineurial fibroblasts or both. Here, we describe the histologic and immunohistochemical features of a recurrent MPNST in a hedgehog.

Materials and Methods: A three year-old female african hedgehog developed a recurrent mass on vagina. Tissue sample were collected, fixed in 10% neutral-buffered formalin, embedded in paraffin and sectioned at 4 µm for hematoxilin and eosin staining. An immunohistochemical panel consisting of vimentin, alpha-smooth muscle actin (αSMA), glial fibrillary acid protein (GFAP), neuron-specific enolase, CD10, and S100 protein was applied.

Results: The histopathologic findings were high cellularity, with a variable pleomorphic, plump spindle cells arranged mostly in interlacing bundles, with bizarre mitotic figures. Eventual areas of palisading and whorls were observed. The tumor cells were positive for vimentin, enolase, S100 and CD10 with negativity for αSMA and GFAP. The association of morphologic and immunohistochemical characteristics were consistent with the diagnosis of MPNST. Despite the high number of tumors in hedgehogs, this is only the second report of a vaginal MPNST tumor in an african hedgehog.

Conclusions: The histogenesis of soft tissue tumors can be difficult to differentiate on the basis of routine histologic analysis. The immunohistochemistry is an adequate complementary method for the differential diagnosis of MPNST, with an important cross-reactivity of some human antibodies with hedgehog tissues.

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URACHAL CARCINOMA IN A YOUNG ADULT DOG

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Introduction: Tumours arising from embryonal remnants are rare, among these urachal carcinoma (UC) are sporadically reported in humans, mostly in adult and aged patients, and less frequently in children and adolescents. To our knowledge UC has never been reported in dogs.

Materials and Methods: A 18 month-old, male, Labrador Retriever dog was referred for abdominal pain. Ultrasounds revealed multiple large (up to 25 cm) cystic abdominal masses, adherent to the omentum, and close to pancreas and spleen. The largest mass was surgically removed and formalin fixed for histology. Immunohistochemistry with antibodies anti-cytokeratin and uroplakin III was also performed. Computed Tomography examination didn't reveal findings compatible with metastatic lesions. A postoperative chemotherapy switch was performed.

Results: Histologically a large, multinodular, partially capsulated, infiltrating, cystic neoplasm, involving mesenterial fat was observed. The neoplasm was composed of small cystic structures lined either by simple columnar epithelium with intermingled goblet cells (intestinal type) or by pseudostratified epithelium with 4-5 rows of nuclei and occasionally superficial luminal umbrella cells (urothelial type). Immunohistochemically all neoplastic cells were intensely, diffusely cytokeratin positive. In urothelial type areas neoplastic cells also showed intense intracytoplasmic expression of uroplakin III. Twelve months after surgery the dog is still alive in very good clinical condition without relapse.

Conclusions: UC is a rare entity in human medicine characterised by double intestinal (colonic) and urothelial differentiation. Metastatic cases are reported, with variable fates. The present case is the first report of UC in dogs. The diagnosis was possible based on both histology and immunohistochemistry.

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CYSTIC LESION IN THE ADRENAL GLAND OF A BEAGLE DOG

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Introduction: Cystic lesions of the adrenal gland are uncommon in man and only a few cases have been reported. In domestic animals, the occurrence of epithelial cysts has been reported in the adrenal glands of non-human primates but never reported in the dog.

Materials and Methods: In the context of a chronic repeat dose toxicity study, a single spherical cystic lesion, 1 mm in diameter, was observed in the cortex of one adrenal gland from a male Beagle dog. No clinical signs or other relevant gross changes were recorded for this dog. Histological analysis, special stains and immunohistochemical analysis were performed in order to characterize the lesion.

Results: Histologically, the cyst was lined by a stratified epithelium composed of cuboidal to columnar cells, occasionally vacuolated. The cyst was filled by moderate amounts of pale eosinophilic amorphous material and red blood cells. PAS and Alcian blue positive material was present in the cytoplasmic vacuoles. Epithelial cells expressed CK 19 and CK 20.

Conclusions: The histological, histochemical and immunohistochemical analysis suggested an intestinal origin of this adrenal cyst. The lesion was interpreted as an intestinal choristoma. The occurrence of intestinal choristoma in the dog has been reported as a single instance only in the subcutis of the flank.

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191 A CASE REPORT OF HEPATIC MYELOLIPOMA IN A FERRET (*MUSTELA PUTORIUS FURO*)

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Introduction: Myelolipoma is a rare mesenchymal benign tumour primarily composed of mature adipose tissue with scattered hematopoietic elements in different proportions. Myelipomas are typically non-functioning tumours and therefore often asymptomatic. Several cases of myelolipoma have already been reported both in humans and animals, but so far only a splenic myelolipoma has been reported in ferret.

Materials and Methods: A 6-years-old neutered male white ferret (*Mustela putorius furo*) was referred with sudden depression and anorexia. Abdominal ultrasonographic investigations revealed two hepatic masses, which were surgically removed. Specimens were fixed in 10% phosphate-buffered formalin for histological examinations.

Results: Histologically, the mass showed mature adipocytes associated with hematopoietic elements, represented by granulocytic, erythrocytic and megakaryocytic series at different stages of maturation. These findings were consistent with a diagnosis of myelolipoma. This is the first report of a hepatic myelolipoma in a ferret.

Conclusions: The pathogenesis of myelolipoma remains unclear: it is considered to be a hormonally induced metaplasia of stromal cells or primitive mesenchymal cells. Another hypothesis is related to a possible activation of dormant hematopoietic stem cells in the peritoneum that had been active during the embryonic development. Myelolipomas are also speculated to be derived from bone marrow emboli lodging in different organs. Others authors suggest that myelolipoma is a choristoma, arising from normal hematopoietic stem cells, misplaced during embryogenesis. The few case reports in veterinary literature suggest that myelolipoma is a disease of the geriatric animal, with a similar scenario to that reported in humans.

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TRANSTIONAL CELL CARCINOMA METASTAZING TO DIFFERENT ORGANS INCLUDING THE EYE IN A CALIFORNIAN SEA LION (*ZALOPHUS CALIFORNIANUS*)

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Introduction: A twenty-five year old male captive California sea lion (*Zalophus californianus*) lost 40kg of body weight in two weeks, suffered from ascites and was diagnosed with a heart murmur. It was decided to euthanize the animal and to perform a necropsy in order to elucidate the cause of disease.

Materials and Methods: A full autopsy on the sea lion was performed with histopathologic examination with additional immunohistochemical staining, such as Low Molecular Weight Cytokeratin (LMWC), High Molecular Weight Cytokeratin (HMWC) and Uroplakin.

Results: Macroscopically, the bladder contained a yellow-white firm nodule (2x3 centimeters). Similar nodules were found in liver and lung. Draining lymph nodes were severely enlarged. This infiltrating nonpapillary neoplasm, organized in lobules and nests with central necrosis and numerous neoplastic emboli in blood and lymphatic vessels stained positive and negative for LWMC and HWMC respectively. Staining with uroplakin confirmed the diagnosis of a transitional cell carcinoma (TCC). The neoplasm showed a similar morphology in other organs, including the eye.

Conclusions: TCCs are a common neoplastic lesion in the urogenital tract of the Californian Sea Lion[1]. Various patterns of TCCs have been described, with the nonpapillary-infiltrating type as the second most common. The latter is known to metastasize, however involvement of the eye has not yet been described. The cause of TCC remains often undetermined, but is likely multifactorial. Environmental contaminants, such as polycyclic aromatic hydrocarbons like benzo-pyrine and polychlorinated biphenyls are known as causative agents. Since the Sea lion was kept in captivity during his live, these contaminants can most probably be excluded.

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REACTION OF COCAINE- AND AMPHETAMINE-REGULATED TRANSCRIPT – LIKE IMMUNOREACTIVE (CART-LI) NERVE FIBERS IN THE WALL OF THE PORCINE GALL BLADDER ON LIPOPOLYSACCHARIDES FROM *SALMONELLA ENTERITIDIS*

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Introduction: Cocaine- and amphetamine-regulated transcript (CART) is a neuronal factor, whose exact functions in gastrointestinal regulatory processes remain unknown. On the other hand, toxic properties of lipopolysaccharides (LPS)- components of Gram-negative bacteria wall are well known, however the influence of them on CART+ nervous structures within porcine gall bladder has not been studied yet.

Materials and Methods: Fragments of gall bladder body were collected from five control pigs (C-group) and from five animals, which seven days earlier received intravenously 5 µg/kg body weight solution of LPS from *Salmonella Enteritidis* (L7770, Sigma). After immersion with 4% paraformaldehyde, tissues were cut on ten-µm-thick cryostat sections and subjected to single-labelling immunofluorescence using anti-CART antiserum. The number of CART+ fibers *per* observation field (0.1 mm^2) was evaluated (50 observation fields in each animal). Data were pooled and presented as a mean±SEM.

Results: LPS administration caused clear, statistically significant ($p \leq 0.05$) increase of the number of CART+ nerve fibers (from 11.11±1.56 to 19.75±0.19). Moreover, after LPS administration CART+ nerves were thicker and built larger bundles in compare to C-group.

Conclusion: CART seems to be an important substance in nerves within gall bladder wall in physiological conditions as well as during LPS intoxication. Observed changes suggest participation of CART in adaptive and/or neuroprotective processes within nervous structures supplying the gall bladder. However, the exact role of CART during bacterial LPS intoxication requires further investigations. *Supported by KNOW (Leading National Research Centre) Scientific Consortium "Healthy Animal – Safe Food", decision of Ministry of Science and Higher Education No. 05-1/KNOW2/2015.*

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THE INFLUENCE OF CHEMICALLY - INDUCED INFLAMMATION ON NITRERGIC NERVOUS STRUCTURES WITHIN THE MUSCULAR LAYER OF THE PORCINE DESCENDING COLON

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Introduction: Myenteric plexus (MP) - responsible for intestinal motility belongs to the enteric nervous system (ENS). One of substances described in the MP is nitric oxide (NO) – gaseous neurotransmitter, whose functions during enteritis are not fully explained. This study describes changes in immunoreactivity to nitric oxide synthase (NOS – nitrergic neurons marker) in cells of MP and nerves in the intestinal muscular layer during colitis.

Materials and methods: Animals were divided into three groups: control (C; n = 5), "sham" operated, (C1-group; n=5) and pigs with colitis (I-group; n=5). Animals of I-group were injected with 80µl of 10% formalin (microinjections per 5-8µl) into colonic wall. C1 pigs were injected in the same manner with a saline solution. After seven days fragments of descending colon were subjected to double-labelling immunofluorescence using anti - protein gene product 9.5 (PGP 9.5 – pan-neuronal marker) and anti – NOS antibodies. The percentage of NOS+ neurons in relation to all PGP 9.5+ cells, as well as the number of muscular NOS+ nerves in observation field (0.1 mm2) were evaluated.

Results: Colitis caused the increase in the number of NOS+ cells in the MP from 11.70±0.94% to 19.04±1.09%. The number of NOS+ nerves also increased (from 19.80±1.24 to 34.60±1,29). Statistically significant differences, by contrast, were no observed between C- and C1-groups.

Conclusion: Results show that NO plays adaptive and/or neuroprotective roles within ENS during inflammatory processes. Supported by KNOW (Leading National Research Centre) Scientific Consortium "Healthy Animal – Safe Food", decision of Ministry of Science and Higher Education No. 05-1/KNOW2/2015.

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INFLAMMATORY BOWEL DISEASE (IBD) AFFECTS DENSITY OF NITRERGIC MUCOSAL NERVE PROCESSES IN THE CANINE GASTROINTESTINAL TRACT

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Introduction: The aim of this study was to determine the number of nitric oxide synthase-positive nerve fibers in the mucosa of different sections of the canine gastrointestinal tract under physiological conditions and in patients with variable severity of IBD.

Materials and Methods: Twenty eight German shepherd hybrid dogs of both sexes, with body weight of 15 to 25 kg, aged 6 to 10 years have been used to the present investigation. All experimental animals were qualified for the experiment on the basis of clinical, laboratory, and endoscopic examination results as well as histopathology of duodenal, jejunal, and colonic mucosa. Fiber density was evaluated in biopsy specimens of duodenal, jejunal and descending colon mucosa obtained from healthy dogs and patients with variable severity of the disease. Immunohistochemical stainings were performed by the immunofluorescence method. The density of NO-immunorecative nerve fibers was determined by the semi-quantitative method by counting fibers in the fields of view (0.I mm^2).

Results: The obtained results show that inflammatory bowel disease causes statistically significant increase of the number of mucosal nitrergic nerve fibers in all intestinal segments studied, and this changes are directly proportional to intensity of the disease process.

Conclusions: Noted proportional changes in density of the NOS-positive nerve fibers to intensity of disease process can be useful indicator in diagnostic evaluation of the stage of IBD in dogs. *Supported by KNOW (Leading National Research Centre) Scientific Consortium "Healthy Animal – Safe Food", decision of Ministry of Science and Higher Education No. 05-1/KNOW2/2015.*

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ALTERATIONS OF SUBSTANCE P (SP) EXPRESSION IN GASTROINTESTINAL MUCOSAL NERVE FIBERS OF THE INFLAMMATORY BOWEL DISEASE (IBD) SUFFERING DOGS

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Introduction: Based on available data SP known as proinflammatory neuromodulator might play a role in the IBD pathogenesis. Therefore the goal of our study was to investigate expression of SP in the intestines of IBD suffering dogs.

Materials and Methods: The study was performed on twenty eight German shepherd hybrid dogs of both sexes, of 15 to 25 kg of body weight. All experimental animals were qualified for the experiment on the basis of clinical, laboratory, and endoscopic examination results as well as histopathology of duodenal, jejunal, and colonic mucosa. Fiber density was evaluated in biopsy specimens of duodenal, jejunal and descending colon mucosa obtained from healthy dogs and patients with variable severity of the disease.

Results: It has been found that IBD induces changes in density of the mucosal SP-immunoreactive nerve fibers. After the initial increase in group of mild IBD patients there was significant decrease in number of SP-immunoreactive nerve fibers in duodenal and small intestine mucosa, while in the colon continuous increase was found. Though, in group of severe IBD patients an increase in density of SP-immunoreactive nerve fibers in all studied segments of the intestinal tract was noted.

Conclusions: Detected proportional changes in density of the of SP-positive nerve fibers in colonic mucosa to the intensity of the disease process can be useful in diagnostic evaluation of stage of IBD in dogs. *Supported by KNOW (Leading National Research Centre) Scientific Consortium "Healthy Animal – Safe Food", decision of Ministry of Science and Higher Education No. 05-1/ KNOW2/2015.*

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ALTERATIONS IN CHEMICAL EXPRESSION OF SOMATOSTATIN IN THE SYMPATHETIC NEURONS SUPPLYING THE PREPYLORIC REGION OF THE PORCINE STOMACH INDUCED BY SELECTED PATHOLOGICAL CONDITIONS

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Introduction: Gastric disorders are among the most common diseases. The present study was designed to define chemical expression of somatostatin in the sympathetic perikarya innervating the porcine stomach prepyloric area in physiological state, during acetylsalicylic acid induced gastritis and following partial stomach resection.

Materials and Methods: The neuronal retrograde marker Fast Blue (FB) was injected into the anterior prepyloric wall of stomachs of control (n=5), acetylsalicylic acid treated (ASA) (n=5) and partial stomach resection (RES) (n=5) groups. Animals in ASA group were given acetylsalicylic acid orally for 21 days. On 22nd day after FB injection, in animals of RES group partial stomach resection was performed. On 28th day all pigs were euthanized. Then cryostat sections were double immunolabeled using anti-somatostatin (SOM) and anti-tyrosine hydroxylase (TH) antibody. As the secondary antibody AlexaFluor 546 and AlexaFluor 488 were applied.

Results: In control group SOM was found in 14.97 ± 1.57 % out of 300 FB- positive CSMG neurons. In experimental groups the growth of population of SOM-IR neurons was observed (to 33.72 ± 4.39 % in ASA group and to 39.02 ± 3.65 % in RES group, respectively). Moreover, all SOM-IR perikarya were simultaneously TH immunoreactive.

Conclusions: These results confirm involvement of SOM in the sympathetic regulation of stomach function in pig. The increase in the number of SOM-IR neurons during selected pathological condition may suggest its neuroprotective function. *Supported by KNOW (Leading National Research Centre) Scientific Consortium "Healthy Animal – Safe Food", decision of Ministry of Science and Higher Education No. 05-1/KNOW2/2015*

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CHARACTERIZATION OF DECIDUAL REACTION IN A SUPEROVULATION PROTOCOL IN C57BL/6J MICE

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Introduction: Bilateral multiple uterine nodules were observed in 31 of 276 (11.2%) young adult C57BL/6J female mice following a protocol of superovulation . These lesions made unable the embryo collection. Uterine decidual reaction was suspected. The aim of this study was to analyze the immunohistochemical pattern of the lesions and the molecular expression of cyclin D3, Hoxa-10 and Heparin-binding Epidermal growth factor-like growth factor (HB-EGF) to elucidate a possible pathogenic mechanism.

Materials and Methods: All animals were injected with 7.5 IU PMSG and 7.5 IU HCG for superovulation. Samples of animals that developed uterine nodules (n=20), 8.4 ± 0.7 weeks-old, and normal animals treated with similar superovulation protocol (control group, n= 10), 8.7 ± 0.5 weeks-old, were collected. Immunohistochemistry was undertaken to evaluate desmin, vimentin, progesterone receptor (PR), estrogen receptor α (ERα), Ki-67, cyclin D3 as well as RT-qPCR to asses cyclin D3, Hoxa-10 and HB-EGF mRNA, were performed.

Results: Uterine decidual reaction presented a high degree of structural organization. Antimesometrial region contained decidual cells, frequently polyploid or binucleated, and immunopositive for desmin. Ki-67 proliferation index, PR and cyclin D3 markers were significantly higher immunoexpressed in decidual cells when compared to stromal cells in pathological and healthy uteri. Significantly higher expression of cyclin D3 and Hoxa-10 mRNA was also observed in pathological uteri.

Conclusions: Our results suggest that PR overexpression in stromal antimesometrial cells, probably due to high progesterone (P4) levels triggers cyclin D3 and Hoxa-10 overexpressions that could be involved in the pathological mechanisms of mice uterine decidual reaction.

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ABORTION CASE IN HANWOO CAUSED BY BOVINE VIRAL DIARRHEA AND BOVINE EPHEMERAL FEVER

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Introduction: The infectious causes of abortion in cattle include various agents such as bacteria, virus, etc. Bovine viral diarrhea(BVD) is frequently diagnosed in abortion cases and Bovine Ephemeral fever(BEF) is also a known abortion-inducing agent.

Materials and Methods: No clinical symptoms were noted before abortion. After postmortem examination, all organs were fixed in 10% neutral buffered formalin and embedded in paraffin wax. The embedded tissues were sectioned and subsequently stained with hematoxylin and eosin.. Genomic DNA and RNA was also extracted from organ samples using QIAamp kit. Multiplex PCR was carried out to detect agents including *Neospora canis, Leptospira* spp, *Campylobacter fetus, Listeria monocytogenes* and *Coxiella burnetii*. Viral agents such as bovine herpesvirus-1&5, BVDV, Ainovirus, Chuzanvirus, Ibarakivirus, bovine ephemeral fever virus and akabanevirus were also examined by PCRmmunohistochemistry using the ABC method. Antigen retrival usedprotease 1(Ventana) and primary antibodies were anti-BVDV antibody 15C5 (Syracuse Bioanalytical) and anti-BEFV antibody Aly(novusbio)

Results: On necropsy no specific gross lesions were found and crown-rump length was 51cm. Histologically, multifocal granulomatous meningoencephlaitis and granulomatous hepatitis was observed . By PCR , all organs were positive for BVDV and brain was also positive for BEFV. By IHC all organs were strongly- positive for BVDV antigen and brain was weakly- positive for BEFV antigen.

Conclusions: In conclusion, we confirmed abortion involving both BVDV and BEFV using diagnostic tools such as histopathological findings, PCR and IHC. This is a first report for BVD and BEF infections in an aborted fetus in Korea.

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CAT OVARY BIOMARKERS

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Introduction: In the ovary, the alternation in dominance between follicles and *corpora lutea* translates into a cyclical dynamics of sex hormones. The understanding of the morphology and immunohistochemical biomarkers was the aim of this study.

Material and Methods: We used 20 sections of ovaries, either in the follicular (n=10) or luteal (n=10) phase of the estrous cycle. This phase was determined based on the morphological aspect of the ovary and uterus; the estrogenic phase presented follicular growth in the ovary and nonspecific glands in the endometrium and the luteal phase having *corpora lutea* in the ovary and endometrium glands at its maximum coiling expression.

Results: The expression of cytokeratins 7 (CK7), 8/18, 14 and 20 for epithelial cells and vimentin, desmin and alpha-actin for stroma, vessels and muscle was performed by indirect immunohistochemistry.

Blood vessels, theca cells and stroma showed positive staining for vimentin, desmin and alpha actin. *Corpora lutea* had strong positivity for vimentina, Ck7 and 20, and weak and inconstant for CK8/18. Surface epithelium showed strong labelling for CK 7, 8/18 and is negative to CK14 and 20. *Rete ovarii* were positive for CK8/18 and CK7. Granulosa cells and interstitial gland had moderate positivity for CK7 and 20.

Conclusion: This is important knowledge on morphological biomarkers of the cat ovary and this can be used to assess other ovarian lesions.

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CHANGES IN NORADRENERGIC AND CHOLINERGIC INNERVATION PATTERNS IN THE OVARIES IN PROGESTERONE-TREATED GILTS

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Introduction: The aim of the study was to determine the effect of long-term progesterone (P4) exposure, a simulation of pathological states that occur with P4 overproduction, on the distribution and number of noradrenergic and cholinergic nerve fibres in the ovaries of gilts.

Materials and Methods: From Day 4 of the first estrous cycle to Day 9 of the third cycle the experimental gilts (n = 3) were injected i.m. with P_4 (100mg every 12h), whereas the control gilts (n = 3) with corn oil. Blood samples were taken every 12h and the concentrations of steroids were determined by RIA. The gilts were slaughtered on Day 48 of the study and ovaries were collected. Serial ovarian sections were subjected to single immunofluorescence staining.

Results: After P4 administration, there were an increase ($P<0.05$-0.001) in P4, androstenedione and 17β-estradiol levels, and a decrease ($P<0.05$, $P<0.01$) in testosterone and estrone levels in the experimental gilts compared to the control. P4 treatment led to an increase in the density of dopamine β-hydroxylase (DβH)-immunoreactive (IR) nerve fibres near primary ($P<0.05$) and medium tertiary ($P<0.01$) follicles and of vesicular acetylcholine transporter (VAChT)-IR nerve fibres in the cortical part of ground plexus ($P<0.05$), near secondary follicles ($P<0.05$) and medullary veins ($P<0.05$). DβH- and VAChT-IR fibers were not found near the corpora lutea in the P4-treated gilts.

Conclusions: Our results suggest that elevated P4 levels occur during pathological conditions and may affect the distribution and number of noradrenergic and cholinergic nerve fibres in ovaries, and their function(s).

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PATHOLOGICAL CHANGES IN THE OVARIES OF AN ANTILLEAN MANATEE (*TRICHECHUS MANATUS MANATUS* L. 1758)

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Introduction: Lesions within reproductive organs can lead to systemic health issues in animals. The treatment of such diseases is very complicated in case of large marine mammals because of their life environment. Here, we aim to expand knowledge about morphology of reproductive organ in marine animals and describe the pathological changes found in ovaries of an Antillean manatee (*Trichechus manatus manatus*).

Materials and Methods: The 23 year-old female Antillean manatee from Wroclaw Zoological Garden died on April 4, 2015. The samples from left and right ovaries were collected and then were stained by H&E and Azan trichrome to evaluate collagen fibres arrangement, the periodic acid-Schiff method to visualise glycans, glycoconjugates and neutral glycoproteins, and by Alcian Blue (pH 2.5) to detect acid sialylated glycosaminoglycans.

Results: Small and large ovarian cysts, surrounded by a connective tissue capsule, were found in samples from the ovarian follicles. Some of these cysts were attached to the ovary by stalks. Several cysts contained a dark pink, uniform mass, while other cysts had a high columnar epithelium containing a weakly positive eosin fluid. Numerous lymphocytes were found within the connective tissue of the cyst capsules. The changes in the Antillean manatee ovaries did not indicate female reproductive tract neoplasia.

Conclusion: This is the first report of ovarian cysts in the Antillean manatee from a zoological garden. Pathological changes of ovaries were not the direct cause of animal's death. The results of this study may be useful in the future in reproductive system diagnostics of marine mammals.

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METABOLIC AND INFECTIOUS DISEASE IN TWO HORNED VIPERS: WHICH CAME FIRST?

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Introduction: Diet and husbandry disturbances easily cause disease in captive reptiles, which become prone to developing additional infectious diseases.

Materials and Methods: Two adult Horned Vipers (*Vipera ammodytes*) were submitted for diagnosis, after sudden death. Necropsy, X-rays, histopathology (H&E, HEA, PAS, Gram) were performed.

Results: The lesions were similar for both snakes. X-rays revealed no lesions. Grossly, in both vipers three parasites belonging to *Filaroidea* superfamily were observed subcutaneously, in the mid third of the body, with no vital reaction. The lung exhibited mild hyperemia, the liver was discretely enlarged and discolored, the kidneys presented subtle white stripes, specific to gout. The intestine was distended by white-gray pasty content and gas, along with a 15 mm long parasite with similar morphology as the aforementioned. The histopathology revealed interstitial pneumonia, type II pneumocyte hyperplasia, severe desquamation of the alveolar epithelium, edema, emphysema and heterophylic infiltration with Gram positive and negative intralesional bacteria for the lung, moderate hepatic lipidosis, mild enteritis with enterocyte desquamation. Additionally, the kidneys exhibited tubular necrosis with gout crystals and cast formation, and congested glomerular capillary; throughout the pancreas foci of coagulative necrosis with mild heterophylic infiltration were noticed, lesions which, together with the ones observed in the lung, have been described for ophidian Paramyxovirus.

Conclusions: The death of the Horned Vipers was produced by metabolic causes such as hepatic lipidosis, gout and enteritis, associated with a pulmonary pathology consisting of a proliferative pneumonia, which complementary to the coagulative pancreatitis have been linked to ophidian Paramyxovirus.

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THE GOBLET CELL-DERIVED SOLUBLE MUCUS COMPONENT CLCA1 ACTIVATES AIRWAY MACROPHAGES IN THE MOUSE

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Introduction: The mucus layer is the first defense line of innate immunity of the respiratory tract. Specific components of this barrier may act in distinct immunologic pathways. CLCA1 which is secreted by airway mucus cells is thought to play an, as yet, undefined role in early immune responses. It modulates cytokine responses and consecutive cellular leukocyte recruitment in a mouse model of acute pneumonia. We hypothesized that CLCA1 activates murine alveolar macrophages to induce cytokine expression, thereby regulating the inflammatory response.

Materials and Methods: Alveolar macrophages isolated from C57BL/6 mice were stimulated with conditioned media from HEK 293 cells expressing CLCA1, its hydrolase-inactive mutant CLCA1EQ or pcDNA as negative or LPS or LTA as positive controls. Select pro-inflammatory cytokines were quantified on mRNA and protein levels by RT-qPCR and cytometric bead array, respectively. Global gene regulations were analyzed by mRNA microarray analysis.

Results: CLCA1, but not its hydrolase-inactive mutant, induced a significant increase in expression of Cxcl-1, Il-1β and Il-6 in airway macrophages on the mRNA level. On the protein level, the induction of CXCL-1, IL-6 and TNFα was also observed. mRNA microarrays identified upregulation of genes relevant in innate immunity.

Conclusions: Secreted CLCA1 appears to act as a signaling molecule that activates alveolar macrophages in a murine model, similar to its human homolog. However, its hydrolase-inactive mutant does not seem to activate macrophages. Our *in vitro* stimulation of this critical immune cell population confirms and may explain CLCA1`s central immune regulatory function in the model of acute pneumonia.

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THE GOBLET CELL-DERIVED CLCA1 PROTEIN – A SOLUBLE MUCUS COMPONENT WITH ANTIMICROBIAL PROPERTIES

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Introduction: CLCA1, a goblet cell derived soluble protein, is highly expressed on mucous membranes in the airways, intestinal tract and uterus with direct contact to bacterial agents. Antimicrobial peptides or proteins are known components of the mucus barrier and play an important role as the first line of defense in innate immunity. Here, we test whether CLCA1 affects bacterial growth *in vitro*.

Materials and Methods: Several potentially pathogenic bacterial species and strains were grown to midlog phase and incubated for 1 to 3 h with conditioned media from HEK 293 cells overexpressing CLCA1, its hydrolase-inactive mutant CLCA1EQ or pcDNA as negative control. Appropriate dilutions were plated on LB agar plates, incubated over night at 37 °C and colony forming units were determined.

Results: Bacterial growth of *Escherichia coli* and *Staphylococcus aureus* was significantly reduced by CLCA1 but not by its hydrolase-inactive mutant. In contrast, neither CLCA1 nor its mutant variant had an effect on the growth of *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Citrobacter rodentium* and *Bacillus subtilis*.

Conclusions: CLCA1, but not its hydrolase-inactive form, seems to have bacteriostatic or antimicrobial effects on specific pathogens *in vitro* as it is known for other mucus components such as cathelicidins and defensins. The mechanism as well as the restriction of the effect of CLCA1 on only a subset of bacteria remain to be established.

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PATHOLOGY, IMMUNOHISTOCHEMISTRY AND VIROLOGY OF BOVINE PAPILLOMATOSIS: COINFENCTION OF PAPILLOMAVIRIDAE AND POXVIRIDAE

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Introduction: The families Papillomaviridae and Poxviridae include a number of species affecting different mammal species and causing skin lesions. This publication describes histological appearance and identifies the epitheliotropic viruses responsible for the generically defined "papillomatosis".

Materials and Methods: Diagnosis was based on clinical signs, histopathology, immunohistochemistry, virology and electron microscopy. Lesions were submitted in 10 % neutral buffered formalin, digitally photographed and stained with H&E. Immunohistochemistry for CD3, Ki-67, MHC-II, cytokeratin, vimentin and disrupted papillomavirus (BPV) were performed. PCRs, followed by mini-array, were performed to detect zoonotic poxviruses and coinfection with bovine papillomavirus types.

Results: Histopathologically, varying degrees of hyperplasia of the epidermis with irregular papillary projections into the dermis was common. In the epidermis moderate to severe acanthosis, mild to severe hyperkeratosis, hydropic degeneration of keratinocyts and many koilocytes with variably sized keratohyalin granules were present. Rare intranuclear inclusion bodies were observed only in the basal cells of the epidermis. Dermis showed mild to moderate hyperplasia of the connective tissue that consisted of blood vessels, fibroblasts, focal hemorrhage and mild infiltration of lymphocytes. BPV was found in the epidermis by immunohistochemistry, while CD3-positive lymphocytes were present in the epidermis and particularly in the dermis. Biomolecular analyses showed, in many cases, a coinfection of Papillomaviridae and Poxviridae.

Conclusions: Our results show that the so-called "papillomatosis" can be the result of multiple viral infections and only a complete diagnosis including biomolecular tests, histopathology and electron microscopy can be conclusive in most of the cases.

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CYTOLOGICAL AND HISTOLOGICAL CHARATERIZATION OF A CUTANEOUS PHAEOHYPHOMYCOSIS IN A CAT

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Introduction: Cutaneous phaeohyphomycosis is heterogenous group of opportunistic mycotic infections caused by pigmented fungi of the following genera: Alternaria, Bipolaris, Cladosporium, Curvularia, Exophiala, Moniliella, Ochroconis, Phialemonium, Phialophora, Pseudomicrodochium, Scolecobasidium and Stemphylium. It usually affects the skin and subcutis, but central nervous system and disseminated infections may also occur. It primarily affects cats, but can also occur in horses, cattle, dogs and birds. Predilection sites in cats are nose and paws.

Materials and Methods: Fine needle aspiration from the lesion was evaluated after staining with May-Grünwald-Giemsa. Histological sample was fixed in 4% neutral buffered formalin for 24 hours, processed routinely, embedded in paraffin, sectioned at 4 µm, stained with hematoxylin and eosin and for the detection of fungi with Periodic acid–Schiff and Grocott-Gomori's methenamine silver stains.

Results: A severe and chronic granulomatous dermatitis with numerous intralesional pigmented yeasts was diagnosed cytologically and histologically in a twelve years old, female neutered, European domestic shorthair cat. The cat presented a black, elevated and partially necrotic nodule on the ventral abdomen. The cat was diabetic. Cytologically, numerous macrophages could be observed with phagocytosed fungi. Histologically, the nodule was characterized by pyogranuloma with many macrophages, multinucleated giant cells and neutrophils. Macrophages and giant cells contain golden-brown pigmented fungal yeast, pseudohyphae and hyphae. They were oval to round, 7-20 um in diameter, with 2-3 um-thick brown walls and central basophilic protoplasm.

Conclusions: Phaehyphomycosis represents a histological differential diagnosis the other subcutaneous mycoses like pigmented eumycotic mycetoma or chromoblastomycosis and a macroscopical differential diagnosis to neoplastic lesions.

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EXPRESSION OF CYTOKERATINS IN NORMAL SKIN AND SCABIETIC LESIONS OF NATURALLY INFECTED SHEEP

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Introduction: Cytokeratins (CKs) are intermediate filament proteins which are markers of epithelial cell differentiation. Considering that epidermal hyperplasia is a main histopathological feature of ovine sarcoptic mange, our study aims to analyze the expression profiles of various cytokeratins in scabietic lesions.

Materials and Methods: Facial skin biopsies of 10 healthy sheep and 20 naturally infected sheep with *Sarcoptes Scabiei* were obtained and fixed in 10% neutral buffered formalin. The expression of cytokeratins CKAE1/AE3, CK34BE12, CKMNF116, CK5/6, CK14 and CK19 was investigated immunohistochemically using cross reacting anti-human commercial antibodies.

Results: In normal skin, CKAE1/AE3 and CK34BE12 were expressed in all epithelial structures. CKMNF116 was expressed in stratum basale and 2-3 layers of stratum spinosum of epidermis, in follicular epithelium and apocrine glands. CK5/6 expression was observed in epidermal stratum basale and 1-2 layers of stratum spinosum and moreover, in outer root sheath of hair follicles and myoepithelial cells of apocrine glands. CK14 expression was observed in stratum basale of epidermis, in outer root sheath of hair follicles and sebaceous glands. CK19 labeling was confined only to apocrine glands. In scabietic skin, CK34BE12 lost its expression in stratum granulosum and stratum corneum, while CKMNF116 was expressed in all layers of stratum spinosum. CK5/6 and CK14 were expressed in most layers of stratum spinosum and inner root sheath of hair follicles. No comparative differences were seen for CKAE1/AE3 and CK19.

Conclusion: Altered expression of certain cytokeratins reflects the occurrence of modifications in the differentiation and proliferation of keratinocytes in scabietic skin.

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CLCA2 IS OVEREXPRESSED IN PSORIATIC BUT NOT IN ATOPIC DERMATITIS MOUSE SKIN MODELS

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Introduction: CLCA2 is expressed in keratinocytes and is thought to be required for epidermal differentiation. Terminal epidermal differentiation proteins like filaggrin have a pivotal role in skin barrier function and are highly regulated in skin diseases. Here, we studied the expression pattern of CLCA2 in murine skin models of atopic dermatitis and psoriasis.

Materials and Methods: Atopic dermatitis or psoriasis-like disease were induced in hairless SKH-1 mice via oxazolone sensitization or in BALB/c mice via topical application of imiquimod, respectively. The expression pattern of CLCA2 was characterized via RT-qPCR and immunohistochemistry in the inflamed murine skin and healthy controls.

Results: Expression of CLCA2 mRNA was approximately 5-times higher in psoriasis-like skin compared to healthy controls whereas it was unchanged in the atopic dermatitis model. This disease-specific regulation was confirmed on the protein level in both models.

Conclusions: CLCA2 is overexpressed only in the psoriatic skin model, but not in atopic dermatitis. This difference is consistent with hyperkeratosis-associated increased expression of other terminal differential proteins in psoriasis in contrast to atopic dermatitis. Our results support the notion that CLCA2 is a terminal differentiation protein.

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ABSCESS FORMATION AFTER INTRADERMAL INOCULATION OF *STAPHYLOCOCCUS AUREUS* IN A RABBIT MODEL

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Introduction: *Staphylococcus aureus* produces suppurative lesions in rabbits of all ages. Clone ST121 is the most widespread on commercial rabbit farms.

Materials and Methods: Thirty rabbits (*Oryctolagus cuniculus*) were intradermally infected with 300 cfu of *S. aureus* ST121 clone, in the lumbar region. Samples were taken at 0, 1, 2, 3, 7 and 14 days post-infection (dpi), sacrificing 5 animals in each sampling time. Blood samples were taken to characterize peripheral cellular immune response by flow cytometry. Injured tissues samples were also taken to carry out histopathological studies. In addition, samples from lesions, blood and kidney were taken for bacterial counts.

Results: All the inoculated animals developed lesions, which started with erythematous papules (1dpi), which increased in size to become 3 cm-diameter abscesses (14 dpi), and generally split and part of contents spilled out. After 3 dpi dermonecrosis appeared in 40% of animals, affecting 100% at 7 dpi. Histologically, hyperaemia and numerous heterophils (1-2 dpi) were observed, which gradually concentrated and were located around the inoculation area to form an abscess from 3 dpi. Studying the immune response revealed marked lymphopenia in peripheral blood (2 dpi-7 dpi), justified especially by the drop in the T CD8+ lymphocyte subpopulation. Conversely, levels of granulocytes in blood started increasing from 2dpi until the experiment ended.

Conclusions: The development phases of natural abscesses caused by *S. aureus* were experimentally reproduced in this work, which could be a valid model to determine the pathogeny of staphylococcal skin infections.

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A CONGENITAL CORNIFICATION DISORDER IN A LABRADOR RETRIEVER CROSS DOG

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Introduction: Congenital cornification disorders in dogs are rare and data about clinical, histopathological and ultrastructural features are lacking.

Materials and Methods: A seven month-old female Labrador Retriever cross dog presented witha pruritic generalized scaling dermatitis and severe hyperkeratosis of all the pawpads observed. Features were observed soon after birth. Skin lesions consisted of linear alopecic hyperpigmented plaques, following the Blaschko's lines, with thick brown scales and clusters of dilated follicular ostia. Frond-like hyperkeratotic lesions of all the pawpads with occasional horn-like projections caused lameness. Physical examination and blood tests, including panels for endocrinopathies, were unremarkable. Formalin and glutaraldehyde fixed skin samples were examined by light and electron microscopy.

Results: H&E stained samples from haired skin showed severe and diffuse parakeratosis distending follicular infundibula, and interfollicular orthokeratotic hyperkeratosis. Severe laminated orthokeratotic hyperkeratosis was present in the H&E sections from pawpads. Ultrastructurally, stratum corneum of haired skin showed retained nuclei in the corneocytes in the follicular lumen and ostia, intercellular membranous material, shorter corneodesmosomes and cytoplasmatic vacuoles. EM of pawpads biopsies showed the presence of the same intercellular membranous material. Congenital follicular parakeratosis (CFP) was considered the most probable diagnosis and long term treatment with oral acitretin led to moderate improvement of skin lesions.

Conclusions: To the best of the authors' knowledge, severe involvement of non haired skin and diffuse orthokeratotic hyperkeratosis of the interfollicular epidermis have not been described in dogs with CFP. However, we cannot rule out that this case might represent a different cornification defect never reported before.

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EPITHELIOGENESIS IMPERFECTA IN DORCAS GAZELLE (*GAZELLA DORCAS*)

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Introduction: Epitheliogenesis imperfecta is a congenital condition characterized with localized or widespread discontinuity of squamous epithelium of the skin and mucous membranes. It has been reported mostly in domestic animals - foals, puppies, kittens, lambs and piglets. The condition appears to be a recesive trait, seen often in cases of inbreeding. This report describes finding of epitheliogenesis imperfecta in three gazelle lambs born in past two years in small herd (two females, three males - of which one alpha male) from the Belgrade Zoo.

Material and Methods: Three gazelle lambs were born during past two years in Belgrade Zoo and died during few hours after birth. A complete necropsies were performed and after the macroscopic examination skin samples were collected for histopathological examination. Tissue samples for histopathological examination were fixed in 10% buffered formalin, dehydrated, embedded in paraffin blocks, and 5 µm thick sectiones were stained with hematoxylin and eosin (H&E).

Results: Macroscopically, reddish, glistering, well-demarcated hairless skin areas on the parietal region of the scull, knee and flank region, distal portions of the fore and hind limbs and dental plate were observed. Microscopically, affected skin areas lacked epidermis, including basal membrane, as well as adnexal structures (hair follicles, sebaceous glands and sweat glands). At the junction between unaffected and damaged skin, the epidermis ended abruptly, with its margins remaining adherent, and on some parts detached from the underlying dermis.

Conclusion: According to available data this is the first described case report of epitheliogenesis imperfecta in Dorcas gazelle (*Gazella dorcas*).

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A CASE OF SARCOPTIC MANGE IN A *HYSTRIX CRISTATA* FROM THE NEBRODI MOUNTAINS (SICILY)

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Introduction: Sarcoptic mange is a zoonotic and highly contagious mite infection caused by mites of genus *Sarcoptes* which affected the skin of mammals. In this report authors describe a case of sarcoptic mange in a *Hystrix critata* from Nebrodi park.

Materials and Methods: A female of *Hystrix critata* which showed a poor nutritional status was found dead in pastures near Pettineo (Messina-Sicily). Gross examination of the carcass was performed and histopathological and diagnostic investigations by skin scraping were made.

Results: The skin was affected by extended phenomena of hyperkeratosis, parakeratosis and desquamation mainly involving the hips and the abdominal wall to the axillary region. In the caudoventral portion of the neck it was also possible to observe a form of pachydermia. Multiple cutaneous abscesses with whitish-yellow necrotic material and crusts were multifocally present. Hyperplasia of the head lymph nodes was also observed. Hystologically the epidermis showed severe irregular hyperplasia and parakeratotic hyperkeratosis with a thick crusts and multifocal areas of erosion and ulceration. Numerous mites were embedded within the crust; multifocal large colonies of bacteria were also present. Moderate and diffuse inflammatory infiltrate mostly composed of lymphocytes and plasmacells were detected in the superficial to mild dermis. The mites were identified as *Sarcoptes scabiei*.

Conclusions: To the author's knowledge this is the first report of sarcoptic mange in a *Hystrix critata*. The animal was found in area where contacts between livestock and wildlife occurred frequently. The transmission of some strains of *Sarcoptes scabiei* between domestic and wild hosts is documented.

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HISTOLOGICAL LESIONS AND CELLULAR RESPONSE IN THE SKIN OF ALPINE CHAMOIS (*RUPICAPRA R. RUPICAPRA*) SPONTANEOUSLY AFFECTED BY SARCOPTIC MANGE

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Introduction: Population dynamics of chamois (genus *Rupicapra*, subfamily Caprinae) can be influenced by infectious diseases epizootics, of which sarcoptic mange is probably the most severe in the Alpine chamois (*Rupicapra rupicapra rupicapra*).

Materials and Methods: In this study, skin lesions and cellular inflammatory infiltrates were characterized in 44 Alpine chamois affected by sarcoptic mange. Dermal cellular response were evaluated in comparison with chamois affected by trombiculosis and controls.

Results: Both in sarcoptic mange and trombiculosis, a significant increase of eosinophils, mast cells, T and B lymphocytes and macrophages was detected. Moreover, in sarcoptic mange significant higher numbers of T lymphocytes and macrophages compared to trombiculosis were observed. Lesions in sarcoptic mange were classified in three grades, accordingly to crusts thickness, correlated with mite counts. Grade 3 represented the most severe form with crust thickness more than 3.5 mm, high numbers of mites and severe parakeratosis with diffuse bacteria. Evidence of immediate and delayed hypersensitivity were detected in all three forms associated with diffuse severe epidermal hyperplasia. In grade 3, a significant increase of B lymphocytes was evident compared to grade 1 and 2, while eosinophil counts were significantly higher than in grade 1, but lower than in grade 2 lesions.

Conclusions: This study demonstrated that an involvement of non-protective Th2 immune response could in part account for severe lesions of grade 3.

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POXVIRUS INFECTION ("TATTOO SKIN DISEASE") IN TWO STRIPED DOLPHINS (*STENELLA COERULEOALBA*) STRANDED ALONG THE ITALIAN COASTLINE

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Introduction: Tattoo skin disease (TSD) is a *Poxvirus*-induced cetacean disease characterized by typical skin lesions. Few pathological descriptions and a limited number of TDS reports are available worldwide. We describe herein the histological, biomolecular and virological identification of TSD in two striped dolphins (*Stenella coeruleoalba*) stranded along the Latium and Tuscany coasts of Italy in 2015 and 2016, respectively.

Materials and Methods: A full necropsy was performed on the two male, juvenile and well-preserved dolphins under study, followed by detailed histopathological and transmission electron microscope (TEM) investigations. DNA extraction from skin lesion samples and PCR amplification of *Poxvirus* DNA polymerase were also carried out.

Results: The first striped dolphin showed wide, coalescing, lightly gray skin lesions with dark edges (tattoos) on the head, while the second dolphin had a single, 2 cm-wide, round, yellowish lesion with a slightly dark edge, affecting the mandibular region. Numerous *Poxvirus*-like particles were observed in both animals' skin samples by means of TEM. In the *stratum spinosum*, a multifocal, severe, hydropic degeneration of the keratinocytes was also apparent, with numerous round, 5-10 µm in diameter, eosinophilic, glassy structures (intracytoplasmic eosinophilic inclusion bodies), compatible with type-B poxviral inclusions ("Guarnieri bodies") being additionally found. The overlying *stratum corneum* was mildly hyperplastic (1.5 times over normal), with heavily hyperpigmented keratinocytes, occasionally hosting Guarnieri bodies. Viral DNA polymerase PCR allowed to confirm the presence of *Poxvirus* in the skin from both dolphins.

Conclusions: To our knowledge, this is the first description of TSD in cetaceans stranded along the Italian coastline.

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HISTOPATHOLOGICAL EFFECTS OF CUMULATIVE CONCENTRATIONS OF BUPNRENORPHINE IN BRAIN, LIVER, KIDNEY AND TESTIS OF RATS

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Introduction: Opiate abuse has been a matter of serious concern in adolescent people. The primary drug used in the treatment of opiate addiction is (the partial μ-opioid receptor agonist) Buprenorphine, which dissociates slowly from the receptors and have long half-lives. Opiates have side effects on various organs. In this survey, the effects of ascending doses of Buprenorphine on male rat tissues were investigated.

Materials and Methods: Forty-two male Wistar rats were allocated into 7 groups (Control and 1-6 Treatments). The rats in 1 to 6 treatment groups received 2, 7, 10, 15, 20 and 24 mg/kg Buprenorphine intraperitoneally every day. After 21 days, rats were euthanized humanly and their brain, liver, kidney and testicles were removed to histopathological analysis.

Results: In brain samples, significant reduction in neuronal and neuroglial cells in CA1 and CA3 areas of hippocampus were seen. In kidney samples hyperemia, tubular necrosis, malpighian corpuscle shrinkage and inflammatory cells infiltration were detected. In testis samples, seminiferous tubules necrosis and significant reduction in germinal epithelium, leydig cells, spermiogenesis index (SI) and tubules differentiated index (TDI) were observed in association with increasing doses of Buprenorphine. No pathological changes were observed in liver samples.

Conclusions: Buprenorphine is a mixed agonist-antagonist opioid with low intrinsic activity and high affinity for the μ-opioid receptor and with no intrinsic activity, but high affinity, for the κ-opioid receptor. In this survey, we have shown that abuse or overdose administration of Buprenorphine can cause pathological lesions in brain, kidney, and testicles..

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DECREASED GFAP EXPRESSION IN ASTROCYTES OF RATS WITH EXPERIMENTALLY INDUCED RHEUMATOID ARTHRITIS AND PERIODONTITIS AND TREATED WITH RESVERATROL, CURCUMIN OR IBUPROFEN

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Introduction: Astrogliosis is a defensive transformation in response to injury and inflammation during which astrocytes undergo a complex morphological and functional remodeling involving hypertrophy and pronounced upregulation of glial fibrillary acidic protein (GFAP). Rheumatoid arthritis (RA) and periodontitis (PE) are both chronic inflammatory conditions in which astrogliosis is extensively found. The aim of this study was to observe the effects of resveratrol (RSV), curcumin (CU) and ibuprofen (IB) on the increased GFAP expression in rats experimentally submitted to PE and RA plus PE models.

Materials and Methods: Male Wistar rats were divided into the following groups: I- AR (induced by intradermal immunizations with type II collagen in incomplete Freund´s adjuvant); II- PE (induced by a ligature for 11 days around the cervix of the mandibular first molars); III (AR+PE); IV (PE+IB); V (PE+RSV); VI (PE+CU); VII (AR+PE+IB); VIII (AR+PE+RSV); and IX (AR+PE+CU). Treatment with RSV (10 mg/kg), CU (100 mg/kg) or IB (2 mg/kg) was made orally for 30 days. On day 31, rats were euthanized and brainstem samples were collected for GFAP immunohistochemistry. Morphometry was performed using Metamorph software.

Results: Treatment with IB, RSV or CU decreased in the same extent GFAP expression comparing to groups I (AR), II (PE) and III (AR+PE). No difference on such expression was found between these groups I, II and III.

Conclusions: IB, RSV and CU exhibited similar effects on reducing astrogliosis (as measured though GFAP expression) in all groups submitted to the experimentally induced inflammatory conditions PE and AR plus PE.

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ACUTE OZONE EXPOSITION IN RATS – CHANGES IN THE EXPRESSION OF NFKAPPAB, NRF2 AND IL10

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Introduction: Ozone is regarded as an environmental health hazard. However, it is also produced by electronic "air fresheners" and ozonating bathtube inserts. The study was aimed to investigate the influence of ozone on the expression of NfkappaB, Nrf2 and IL-10.

Materials and Methods: Adult Wistar rats were used. Three groups (n=10) were treated with ozone (1 ppm) for 3 hours and sacrificed after 2, 24 and 48 hours. One group (n=10) served as control. Samples of the lung and liver were taken for gene expression studies with Real Time PCR. The expression of Nrf2 and NFkappaB, as well as IL-10 were studied.

Results: The expression of Nrf2 in the lungs increased slightly 3 h after exposure and decreased dramatically 24 and 48 h after exposure. In the liver the expression pattern was different – decrease of the expression level 3 h post exposition and increase 24 and 48 h after exposition. Expression of NfkappaB in the lungs increases 3h after exposition and decreases after 24 and 48 h. In the liver expression of NfkappaB decreased slightly after 3 h and increased progressively from 24 to 48 h post exposition. IL-10 expression in the lungs decreased 3, 24 and 48 h after exposition, however, in the liver the expression of IL-10 was increased slightly 3 h after exposition and decrased 24 h after exposure.

Conclusions: Acute exposition to ozone has led to the changes in the expression of NfkappaB (proinflamatory), Nrf2 (antiinflamatory) IL-10, a cytokine with anti-inflammmatory activity.

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EVALUATION OF THE CHICKEN CORNEAS FROM ICE TESTS USING TRANSMISSION (TEM) AND SCANNING (SEM) ELECTRON MICROSCOPES

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Introduction: The Isolated Chicken Eye (ICE) test is an alternative in vitro method of assessment of chemicals likely to cause eye damage. Research on the introduction of a histopathological evaluation as an endpoint in the classification of chemicals is being currently conducted. Ultrastructural methods were used in order to find out more about the nature of changes observed when using light microscopy. This is the first described case of using electron microscopy to evaluate the corneas in the ICE test.

Materials and Methods: The ultrastructural examination of 35 eyeballs was conducted. The corneas were divided into 7 groups (5 eyeballs in each), i.e. treated with physiological salt, 10% acetic acid, imidazole, 5% benzalkonium chloride, butyl acetate, and ethyl trimethylacetate, and non-incubated eyeballs. After the experimental part of the ICE test, samples were fixed in 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4 at 4°C for 24 h and placed in a mixture of 1% OsO4 and 0.8% K4[Fe(CN)6]. Then, samples were processed for transmission electron microscopy or scanning electron microscopy and analysed using JEM-1200EX or JSM-6390LV, respectively.

Results: The ultrastructural examination revealed the presence of various changes (e.g. cell vacuolation, necrosis, swelling of the cellular organelles) in all layers of the corneas. Their severity depended on test item.

Conclusions: Although both TEM and SEM examinations are extremely useful tools in the evaluation of lesions caused by chemicals, they will not come into routine use in ICE tests because of their high costs.

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ZINC PHOSPHIDE POISONING IN A DOG

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Introduction: Metallic phosphides are extremely toxic pesticides, their usage being limited to certified applicators. Data regarding the clinical pathology of zinc phosphide poisoning in dogs is largely incomplete, only a few reports being presented in the past. The present report represents a complete and detailed description of pathological changes in a case of intentional zinc phosphide poisoning in a dog.

Materials and Methods: A 1 year old, male, Belgian Shepherd crossbreed dog with a clean medical history and no observed clinical signs prior to death, was submitted for post mortem examination. The dog was found dead by the owner, and near the body there was a suspect mix of bread, fat and a blackish powder. Complete necropsy and histopathological examination using routine techniques were undertaken less than 24 h after death.

Results: At necropsy, multisystemic necrotic and degenerative lesions in the liver, brain, kidneys, heart and the lungs were observed. Histological exam confirmed the presence of necrotic and degenerative lesions of variable severity in all of the examined organs. The toxicological forensic examination revealed the presence of the phosphine gas in the gastric content and the bait.

Conclusions: In the present report, we showed that zinc phosphide is mainly responsible for systemic acute necrotizing and hemorrhagic type of lesions, mainly targeting endothelial cells with secondary ischemic necrosis in the affected tissues.

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ACUTE ZINC TOXICOSIS IN A DOG WITH A GASTRIC FOREIGN BODY

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Introduction: A 14-years old male dog was referred to the Veterinary Teaching Hospital. The animal suffered four days of progressively more severe vomiting, failing to improve in spite of treatment. On clinical examination, the dog was dehydrated and unresponsive. Mucous membranes were pale and dry and a slight jaundice was observed. Abdominal palpation was markedly painful. Abdominal radiographs showed a spherical radiodense 5 cm foreign object located in the gastric body. Ultrasounds did not reveal any point of dehiscence or perforation, although pancreatic parenchyma was strikingly heterogeneous.

Results: Clinical pathology examination showed markedly regenerative anemia, neutrophilia with left shift, dehydration, marked azotemia, hyperbilirubinemia, marked metabolic alkalosis, hypochloremia, hypokalemia, increased ALT, lactate and amylase. Numerous Heinz bodies, eccentrocytes and target cells were observed in blood smears. After treatment with IV fluids, a gastrotomy was performed. The animal died a few hours after the surgery. At necropsy there was a marked catarrhal gastritis, peripancreatic fat necrosis, hepatomegaly and kidneys were yellowish and increased in size. Histopathology showed severe diffuse peripancreatic fat necrosis, necrosis and microvesicular degeneration of acinar pancreatic cells, diffuse hepatocellular vacuolar degeneration and severe diffuse tubular degeneration with numerous hyaline and hemoglobin casts in the kidney.

Conclusions: This case demonstrates the typical clinical, clinicopathological and pathological findings in acute zinc toxicosis in small animals. This diagnosis is rare in Europe and is mostly related to the ingestion of zinc-containing toys or foreign bodies. Zinc serum levels were fourfold higher than referenced values in this animal.

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EVALUATION OF HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL EFFECTS OF METFORMIN HCL-LOADED TWO BEADS FORMULATIONS IN STZ-NICOTINAMIDE INDUCED DIABETIC RATS

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Introduction: The main object of this study was to investigate the early stage histopathological and immunohistochemical effects of metformin HCl (MHCl) and MHCl-loaded Alginate (AL) and AL-Chitosan (CS) beads on the pancreas tissues of streptozotocin (STZ)-nicotinamide (NA) induced Type 2 diabetic rats.

Materials and Methods: For this study, a total of 42 rats were divided in 6 groups as control (Goup I), STZ-NA induced diabetic rats (Group II; the fasting blood glucose level >126 mg/dl), STZ-NA induced diabetic rats treated with pure drugs (Group III), blank bead formulation (Group IV), MHCl-loaded-AL-CS (Group V) and AL (GroupVI) bead formulations. The prepared samples of pancreatic tissues were evaluated by histopathological and immunohistochemical methods

Results: Severity of atrophic appearance of pancreatic Langerhans islet was high and frequently observed histopathologically in Groups II, III and IV. The degree of necrotic and degenerative changes of islets of Langerhans in the diabetic rats treated with MHCl-loaded AL-CS and AL beads were reduced compared to the degree of these changes in islets of the other groups. In immunohistochemically, shrunken, distorted islet of langerhans with marked loss immunopositive ß cells was observed for Groups II, III and IV. However, there were improvements in the number of immunopositive ß cells and the diameter of islet of langerhans of diabetic rats treated with drug-loaded AL-CS and AL beads.

Conclusions: Metformin HCl-loaded AL-CS and AL beads might be benifical and effective in reducing the histopathological changes of pancreas and increasing the number of immunopositive β cells, and thereby to reduce the effects of diabetes.

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GFAP IMMUNOREACTIVITY IN THE MESENCEPHALON AND NUCLEUS ACCUMBENS OF RATS TREATED WITH DIFFERENT CLASSES OF NEUROPATHIC PAIN RELIEVERS

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Introduction: A wide range of drugs such as antidepressants, anticonvulsants and opioid analgesics are frequently used in situations of acute, chronic and neuropathic pain. Although pain is considered traditionally as mediated by neurons, recent research shows an important role of glial cells in persistent pain sensitization, especially microglia and astrocytes. The aim of this study was to determine, after the administration of short-term doses of some neuropathic pain relievers, the astrocyte behavior in the mesencephalon and nucleus accumbens (NAc) through the expression of the astrocytic biomarker GFAP (glial fibrillary acidic protein).

Materials and Methods: Male Wistar rats were divided into 5 groups, receiving for 9 days- (1) amitriptyline (Amt- 10 mg/kg/day, by gavage); (2) gabapentin (Gb- 60 mg/kg/day, by gavage; (3) methadone (Me- 4,5 mg/kg/day, intraperitoneal route-IP); (4) morphine (Mo- 10 mg/kg/day, IP); or (5) 0.9% saline solution, IP. Mesencephalon and NAc samples were collected for GFAP immunohistochemical study and the area of GFAP-positive cells was calculated using Metamorph software as total pixel counts.

Results: In the mesencephalon, the expression of GFAP was decreased in the groups treated with Amt, Gb, Me and Mo in relation to controls. As for the NAc, GFAP was increased in the groups treated with Amt, Me and Mo, but not in the Gb-group.

Conclusions: All drugs seemed to modify astrocytic expression of GFAP, but the effect varied according to the region observed. Amt, Gb, Me and Mo decreased this expression in the mesencephalon, while Amt, Me and Mo increased it in the NAc.

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POLYCHLORINATED BIPHENYLS IN THE MUSCLES OF WILD BOARS FROM POLAND

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Introduction: Polychlorinated biphenyls (PCBs) are representatives of a large group of chlorinated hydrocarbons, which due to their environmental persistence, possess the ability to accumulate in the food chain, mainly in adipose tissue. The exposure to PCBs may result in health problems including adverse effects on the nervous, immune, endocrine and reproductive systems, including pathomorphological changes. The objective of the study was to determine the content of polychlorinated biphenyls in the intramuscular fat of wild boars from different regions of Poland.

Materials and Methods: The research material consisted of 47 samples of wild boars muscles from selected regions in Poland: Warmia and Mazury, Silesia and Podlasie. The fat was extracted from the muscle samples and chromatographic determination of congeners PCB was carried out with an Agilent Technologies 6890N with electron capture detector (ECD).

Results: PCBs were found in all tested samples. The average content of the sum of the indicator congeners of PCB (28, 52, 101, 138, 153, 180) in wild boars muscles was 10.25 µg/kg of fat (ranged from 5.30 to 15.37 µg/kg of fat). Statistical differences were found between the muscle samples from different regions.

Conclusions: PCBs were detected in all tested muscle samples, but in none of them were acceptable concentration limits exceeded. Therefore contents of PCBs in wild boars intramuscular fat do not pose a hazard.

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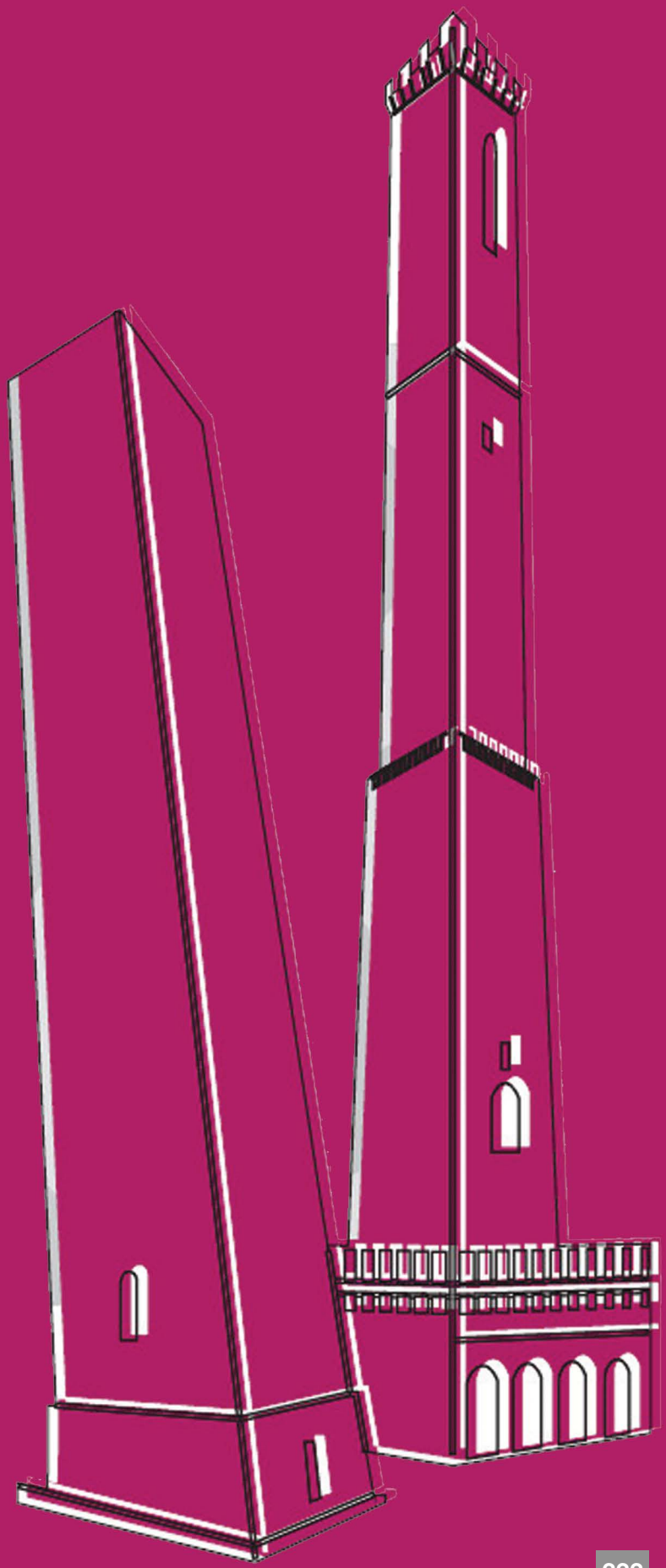

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