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Leibniz Institute for Zoo and Wildlife Research  
(IZW)  
Berlin, Germany  
&  
European Association of Zoo and Wildlife Veterinarians  
(EAZWV)  
Liebefeld-Berne, Switzerland

**PROCEEDINGS OF THE  
ZOO AND WILDLIFE HEALTH  
CONFERENCE  
2017**

May 24<sup>th</sup> – 27<sup>th</sup>, 2017  
Berlin / Germany

Edited by Gábor Á. Czirják  
Josepha Prügel

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ISSN 2510 - 4683

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The contributions included in this volume were carefully checked and revised. Nevertheless, authors and editors are unable to guarantee the correctness of all presented data, conclusions and advice and do not accept liability for possible printings errors. The editors gratefully acknowledge the willingness of the following colleagues for reviewing the manuscripts submitted for this conference:

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**This is also the continuation of the “Proceedings of the International Conference on Diseases of Zoo and Wild Animals”, the 7<sup>th</sup> “Proceedings of the Meeting of the EAZWV” (2008) and the “Erkrankungen der Zootiere – Verhandlungsbericht des 43. Internationalen Symposiums über die Erkrankungen der Zoo- und Wildtiere“ (2007).**

Published by the Leibniz Institute for Zoo and Wildlife Research (IZW)  
Alfred-Kowalke-Str. 17, 10315 Berlin (Friedrichsfelde)  
Postfach 70 04 30, 10324 Berlin, Germany

Printed on FSC-certified paper. The paper has been harvested, processed and manufactured in a sustainable fashion. The Forest Stewardship Council (FSC) label is the gold standard in forest management and sustainable wood products.

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Setting and layout:	Josepha Prügel, Leopold Preuß, Utpala Sharma, Anke Schumann Berlin, Germany
Photo cover and next page:	Western lowland gorilla ( <i>Gorilla gorilla gorilla</i> ) “Ivo” R. Gralki / Zoo Berlin
Printing:	LASERLINE Druckzentrum Berlin KG, Berlin, Germany
Order:	Leibniz Institute for Zoo and Wildlife Research (IZW) Forschungsverbund Berlin e.V. Postfach 70 04 30, 10324 Berlin, Germany <a href="http://www.izw-berlin.de">www.izw-berlin.de</a>

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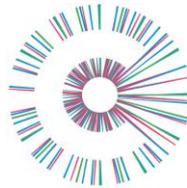
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**ACKNOWLEDGMENT – SPONSORS**

**We wish to thank the supporters of the conference:**



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## **THE ROLE OF (WILD) ANIMALS IN EMERGING VIRAL ZOOSES**

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Outbreaks of emerging infectious diseases can have important human health and economic impacts. A large number of these diseases are zoonotic. Due to changes in the environment, human behaviour and habitat, wildlife is more often seen as a primary source, either directly or via intermediate hosts. Wild birds and bats are species that are well known for spreading emerging zoonotic viruses. Wild migrating birds recently have become important in intercontinental spread of highly pathogenic avian influenza viruses such as H5N8. Some avian influenza viruses spread from domestic birds to humans, causing respiratory disease. Another wild animal group of concern are bats. Bats may harbour many viruses, of which a high proportion may be zoonotic as is demonstrated by the emergence of Ebola virus disease and coronavirus infections such as Middle East respiratory syndrome (MERS) and severe acute respiratory syndrome (SARS). Therefore, monitoring (zoonotic) viruses from wildlife is important to assess the dynamics and the public health hazards of such viruses in order to set up a rapid response system.

## THE PATHOLOGY OF INVASIVE COLONIC ENTAMOEBIASIS, AN EMERGING DISEASE OF CANE TOADS IN AUSTRALIA

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In Australia, cane toads (*Rhinella marina*) have been rapidly expanding their range across the continent since their introduction in 1935. Investigations into natural sources of death in cane toads have identified only starvation and mucormycosis as likely causes. In August 2014, numerous dead and emaciated, moribund toads were found at a research station in tropical Australia. Post-mortem investigation of 22 toads revealed severe invasive colonic amoebiasis in all but one toad. Intestinal amoebiasis has not previously been recognised as a significant cause of mortality in wild amphibians, and while it is suspected as an occasional cause of morbidity in captive amphibians, the associated pathology has not been described.

Affected toads had prominent colonic serosal vasculature, and watery, serosanguineous to overtly haemorrhagic content and segmental to diffuse mural thickening of the intestine. Histologically, the mucosal epithelium varied from hyperplastic (with multiple layers of jumbled undifferentiated cells) to attenuated (with a single layer of undifferentiated cells) to ulcerated. The lamina propria exhibited fibrosis and moderate to marked lymphohistiocytic and lesser granulocytic infiltrate. Amoebae consistent with *Entamoeba* sp. trophozoites were intermingled with epithelial cells, often in clusters aligned along the basement membrane or within tags of sloughing epithelium, and only rarely within the lamina propria. Next-generation sequencing of colon samples confirmed the presence of a genetically distinct species of *Entamoeba* in all affected toads. This genotype was also detected in histologically normal toad colons, indicating that the pathogenesis of invasive entamoebiasis in toads is likely complex, as it is in snakes and humans.

## A NOVEL NIDOVIRUS IN GREEN TREE PYTHONS (*MORELIA VIRIDIS*) AS THE CAUSATIVE AGENT OF MUCOID PNEUMONIA

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We recently observed a noticeable increase in diagnostic necropsy submissions of green tree pythons (*Morelia viridis*) that had shown respiratory distress or had died suddenly. Affected animals exhibited an accumulation of mucus within airways and lung, and a thickening of the entire lung. Here we report the pathological and virological features of "mucoid pneumonia" cases and discuss the pathogenesis of the disease.

Full necropsy and histological examination including immunohistology (IH) was performed on 12 diseased *Morelia viridis* from seven different breeders and four animals without pulmonary alterations (controls). For identification of a causative agent, next generation sequencing (NGS), quantitative RT-PCR (qRT-PCR), virus isolation and transmission electron microscopy (TEM) were employed.

The lungs of all affected animals exhibited mucus accumulation in the lumen and epithelial hyperplasia, with evidence of increased mucus production and chronic inflammation. By NGS, a full length nidovirus genome was retrieved from supernatants of tissue cultures inoculated with lung homogenate of a diseased snake; qRT-PCR, RNA-in situ hybridisation and IH served to demonstrate viral RNA and protein in the airway and lung epithelium of affected snakes. TEM showed nidovirus particles in occasional epithelial cells. Routine bacteriology did not yield pathogenic bacteria.

The study provides strong evidence of a causative relationship between nidovirus infection and chronic hyperplastic mucoid pneumonia in tree pythons. Closely related novel nidoviruses have recently been reported also in ball pythons, Indian pythons and wild shingleback lizards with respiratory disease. This suggests that nidoviruses are emerging or underdiagnosed infectious agents in reptiles.

## MANAGEMENT OF A TULARAEMIA OUTBREAK IN RED TAILED GUENONS (*CERCOPITHECUS ASCANIUS*)

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Tularaemia is a highly infectious and reportable zoonosis caused by *Francisella tularensis*. Increasing frequency of infections in humans and wildlife have been reported in the Netherlands. Non-human primates housed in outdoor enclosures are susceptible to *F. tularensis* infections and contact with infected rodents or hares may be a route of transmission.

At AAP, Rescue Center for Exotic Animals, an adult red-tailed guenon died after non-specific symptoms. Pathological examination revealed granulomatous lesions in multiple organs. Bacteriological culture and PCR of lung material revealed the presence of *F. tularensis* subsp. *holarctica*. In cooperation with veterinary and human health authorities, a plan was made to assess the potential risks for animal and human health. Faecal samples from contact primates and organs of trapped rodents were analysed by PCR and tested all negative. A few days later a group member of the guenon was lethargic and paired serum samples showed an increased antibody titre for *F. tularensis*, indicating active infection. PCR of a rectal swab was positive for *T. tularensis*, suggesting that transmission from this individual to other primates and humans could not be excluded. Despite antimicrobial therapy and initial recovery the guenon died six weeks later. The pathologic findings were similar the first guenon and infection with *F. tularensis* by PCR of lung and spleen was confirmed. The source of this outbreak remained unclear, but transmission by infected rodents or arthropods seems the most likely route. Clinical recognition of tularaemia is essential for prompt preventive measures and appropriate antimicrobial treatment. Primate to primate transmission should be considered.

## **YERSINIA SPP. INFECTION IN ZOO ANIMALS: A RETROSPECTIVE STUDY OVER SEVEN YEARS IN MULHOUSE ZOO**

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Yersiniosis is a prevalent disease in animal collections and is part of risk assessment for zoonotic diseases in zoos. Clinical signs depend on the *Yersinia* species, biotype or serotype and the animal species involved.

Since 2014, isolation of *Yersinia* in routine zoo monitoring led to an increased sampling associated with control measures. Treatment (Trimethoprim-sulfamid, posology depending on the species, Septotryl<sup>ND</sup>) was implemented following antibiotic susceptibility. Testing was performed ten days after the end of treatment and results were negative.

A retrospective study was also conducted to assess the prevalence and importance of *Yersinia spp.* 736 stool samples from 163 animal species were collected over a 7 year period and analysed following the same microbiological process: faeces samples were plated onto *Yersinia* selective agar (CIN-agar, Oxoid) on receipt and after 15 days of cold enrichment. Cultures were incubated at 35 °C for 48 h. Suspicious colonies were identified by MALDI-TOF MS according to the manufacturer's instructions.

Over this period of time, *Yersinia* strains were isolated in 41 samples. Five different *Yersinia* species were involved: *Y. enterocolitica* (36), *Y. frederiksenii* (2), *Y. pseudotuberculosis* (1), *Y. intermedia* (1) and one other *Y. sp* was not identified. 13 strains of *Y. enterocolitica* were sent to the National Reference Laboratory for characterisation. All were from biotype 1A and belong to 4 serotypes: serotypes: O:7,8-8-8,19, O:6,30-6,31, O:34 or non-agglutinable. Those 13 strains were considered non-pathogenic for humans.

An analytical and descriptive epidemiological analysis was conducted. The results of this study show an increase in prevalence during winter months, in 2014 and following years, and a higher infection rate in carnivores. All these findings can be linked to rodent presence in the zoo.

## PUTATIVE RISK AND PROTECTIVE FACTORS ASSOCIATED WITH *TOXOPLASMA GONDII* INFECTION IN CAPTIVE FELIDS

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Cases of toxoplasmosis have been reported in several felid species kept in captivity. This study aimed to determine the seroprevalence for *Toxoplasma (T.) gondii* and putative risk or protective factors focusing on ten small felid species covered by the European Endangered Species Programs (EEP), European Stud Books (ESB) or monitored species within the European Association of Zoos and Aquaria (EAZA).

After validation in domestic cats, *Dipetalogaster maxima*-based blood collection was applied for the majority of samples collected between 2011 and 2013. Samples were tested for antibodies to *T. gondii* by immunofluorescent antibody test and immunoblot. To reach optimal specificity, all animals with non-equivocal results in these tests were excluded. A total of 51 zoos and 311 individuals were enrolled.

Animal (species, sex, age) and zoo specific data (food, housing, hygiene and general health) were collected. Bivariate multi-level modelling and Principle Component Analysis were performed to select variables with the highest biological relevance. Finally, a multivariate multi-level modelling was applied.

Overall 63.0 % of individuals showed *T. gondii* positive serological results. High seroprevalences were found in Pallas' cats (90.4 %) and Rusty-spotted cats (96.4 %), contrasting seroprevalences in Geoffroy's cats (48.5 %) and Margays (47.4 %) which were much lower. The final model revealed that feeding frozen food, avoiding entrance of *T. gondii*-intermediate hosts and high hygienic standards seemed to have protective effects. In general, the effect of age dominated. Seroprevalence in felids  $\geq$  5 years was higher (72.8 %) than in younger ones (44.8 %). This suggested a high rate of postnatal infection in captive felids.

## HAEMOSPORIDIAN PARASITES IN STRIGIFORMES: RESULTS OF AN EPIDEMIOLOGICAL SURVEY IN 15 FRENCH ZOOS

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A limited number of studies about haemosporidia infections in birds have been published. However, these infections may cause major damage to avian populations and represent a concern for veterinarians working in zoological parks or wildlife rescue centers.

Following the loss of 9 Great grey owls (*Strix nebulosa*) at Mulhouse zoo in the summer 2013 and the summer 2015, a prospective epidemiological investigation was performed in captive strigiformes in France. The purpose of this survey was to estimate the current prevalence of haemosporidia infection in strigiformes in French zoos. The objective was also to estimate the prevalence of infection during the nesting period and its repercussion for young birds.

Fifteen zoos participated to the study. Blood samples (0.3 mL of blood collected from the brachial vein) were collected from 122 birds representing 12 strigiforme species. Thin blood smears examination and *cyt b* PCR on EDTA blood allowed us to identify haemosporidian parasites from 44 birds from 10 zoos. Three different species of *Haemoproteus*, and one species of *Plasmodium* were detected. The percentage of birds infected by *Haemoproteus* varied according to the period of sampling. Prevalence during the nesting season (May/June) seemed to be higher with a mean value of 53.9 % (n = 63) compared to the winter season with a mean value of 14.8 % (n = 122). Considering *Plasmodium* infection in strigiformes, the prevalence did not exceed 8 % throughout the year.

This study showed how common haemosporidian infection can be in captive strigiformes in France.

The nesting season was identified as a period of higher risk of infection ( $X^2 = 35.65$ ;  $p < 0.01$ ) and consequently the best period to apply prophylactic measures (disinsectisation, antimalarial treatment).

## USING BEHAVIOURAL ENRICHMENT TOYS TO NON-INVASIVELY MONITOR PATHOGEN SHEDDING IN ZEBRAS (*EQUUS ZEBRA*, *EQUUS QUAGGA*, *EQUUS GREVYI*)

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Diseases pose a significant risk for wild and captive animal populations. Monitoring pathogens in most cases involves physical and chemical restraint with associated inherent risks to animals and veterinarians alike. Therefore, developing alternative non-invasive methods are becoming increasingly important. We aimed to develop a simple method for collecting host and pathogen DNA using an enrichment toy for horses *Snak-a-ball*® (*Likit*™ Talisker Bay, Kirkmichael Azrshire, United Kingdom). The method was tested on mountain zebras (*Equus zebra*; one stallion, five mares), plain zebras (*Equus quagga*; one stallion, three mares, one foal) and Grevy's zebras (*Equus grevyi*; one stallion, three mares, one subadult male) in the Tierpark, Berlin. The animals were vaccinated once in 2011 for Equine herpes virus -1 and -4. The experiment was repeated 19 times (Mountain zebras = 8, Plain zebras = 7, Grevy's zebras = 4). The toy was filled with food pellets and was placed in the enclosures for up to 24 h. During rainfall toys were retrieved as water would reduce DNA recovery. After retrieving the toy, swabs were taken from the surface and DNA was extracted immediately. The focus was on recovering Equine herpes virus and zebra mitochondrial DNA. We identified four different equine herpesvirus strains (EHV); EHV-1, EHV-7, Zebra herpesvirus, Wild ass herpesvirus and zebra mitochondrial DNA from seven individual zebras as potential virus shedders, by PCR and sequencing of amplified products.

The successful detection of pathogens and individual zebras interacting with the toy suggest this non-invasive approach will be useful for health monitoring in captive wildlife and for both population genetics and health studies in free-ranging wildlife. Given the wide variety of enrichment objects that have been developed, this method should be transferable to other species.

## ANTIMICROBIAL BACTERIAL RESISTANCE IN PUBLIC AQUARIA: SHOULD WE BE CONCERNED? PRELIMINARY REPORT CONCERNING SIX YEARS OF BACTERIAL CULTURES AND RESISTANCE TO ANTIMICROBIALS IN FISH, SEA BIRDS, AND AMPHIBIANS

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Bacterial resistance to antimicrobials is an emerging problem, not limited to food industry or hospital infections, but also identified in wild ecosystems even in the most isolated places. Zoo and aquarium veterinarians have been key players in this problem since they are responsible for prescribing antimicrobials in zoological collections and in wild animals.

Bacterial population and pattern of antimicrobial resistance were monitored in the animal collection of Oceanário de Lisboa. In this preliminary report, with data obtained between 2010 and 2015, we considered three taxonomic groups: fish, sea birds and amphibians.

Bacterial cultures were performed under the Health Monitoring Program, from sick animals and primarily at necropsies. Two hundred and ninety seven bacterial isolates were cultured (fish n = 134, seabirds n = 49, amphibians n = 114) and the antimicrobial susceptibility was determined by disk diffusion.

Isolates with the highest antimicrobial resistance were found: in fish – *Pseudomonas putida* was resistant to 87 % of the tested antimicrobials and *Vibrio metschnikovii*, *Morganella morganii*, *Providencia rettgeri* were resistant to 80 %; in seabirds – *Enterococcus faecalis* (93 %) and *Pseudomonas aeruginosa* (89 %) were the most resistant; in amphibians – *Brevundimonas vesicularis*, *Escherichia coli*, *Serratia marcescens*, *Listeria grayi* recorded 89 % while *Microbacterium* spp., *Serratia marcescens*, *Enterobacter cloacae* were resistant to 86 % to the tested antimicrobials.

The average antimicrobial resistance profile was 37.8 % in fish, 59.7 % in seabirds and 64.8 % in amphibians.

This data allowed us to detect bacterial diversity evolution in different animal groups over time and compare patterns of antimicrobial resistance of different isolates, some with a multiresistant profile.

The average antimicrobial resistance profile peaked during 2011 and 2013 and decreased in the last two years. The same general trend occurred with the isolates with multi a resistance profile.

## VERTICAL TRANSMISSION OF ARENAVIRUSES IN BIBD DISEASED BOID SNAKES

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Boid inclusion body disease (BIBD) is one of the infectious diseases threatening captive boid snakes worldwide. It is causally linked to (co-)infection with several reptarenavirus species. Although animals with BIBD often die, mainly due to secondary bacterial or protozoal infections, some chronically reptarenavirus infected animals survive for long periods and can produce offspring. This raised the question, whether parental reptarenavirus infection and/or co-infection with multiple reptarenavirus species can be passed on to the offspring.

To study vertical transmission, we analysed *Boa constrictor* clutches from different breeders with BIBD-positive parental animals and their offspring (in total 10 embryos, 5 perinatal abortions, 24 juveniles). Histology, immunohistology (IH) and transmission electron microscopy (TEM) served to demonstrate the pathognomonic inclusion bodies (IB, consisting of reptarenavirus nucleoprotein) in tissues, and next generation sequencing (NGS) on parental tissues and embryo-derived cell cultures as well as virus-species specific RT-PCR to demonstrate viral nucleic acids.

NGS showed that the "reptarenavirome" of the five clutches studied each comprised several reptarenavirus species and species-specific RT-PCR confirmed the vertical transmission of several or all of these to the offspring. While all embryos were IB free, IB formation was detected in one perinatal abortion and one 2-month old juvenile, then consistently in the juveniles aged eight months or older which indicates postnatal progression of reptarenavirus infection to BIBD. We could also demonstrate the IB i.e. viral antigen in testicular germ cells, the ovarian stroma and the uterus, suggesting that virus transmission to the offspring might occur via germ cells and the placenta.

## LIMITING TRANSMITTER ASSOCIATED MORBIDITY AND MORTALITY IN SNAKES

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### Summary

Forty-seven timber rattlesnakes (*Crotalus horridus*) had 71 radio transmitters implanted surgically in the coelom during a five year study period (2007-2011) assessing habitat utilisation in southern Indiana hardwood forests. An additional 6<sup>th</sup> year of movement data and hematology collection allowed for mortality assessment of the snakes after the final year of surgeries. No acute mortality was noted and only a single overwintering mortality occurred. Regional nerve blocks were performed prior to surgery. Antibiotic injections were provided to all snakes during 48 hours in captivity post-operatively. Geographic information system (GIS) movement data was collected and retrospectively, 22 snakes that received a replacement transmitter were compared one and two week(s) pre- and post- surgery and found no statistical difference. Two post-surgical infections were observed yet both snakes survived and were identified alive outside their hibernacula the following spring.

### Introduction

One component of a multi-agency 100-year Hardwood Ecosystem Experiment involving native mammals, birds, insects, trees, other plants, reptiles and amphibians included gaining information on habitat use, home range size, and hibernacula sites for timber rattlesnakes (*Crotalus horridus*), an endangered species in Indiana. During a six year study period, radio telemetry was used after surgically implanting coelomic radiotransmitters (Holohil Systems Ltd., Carp, Ontario CAN) to investigate how timber rattlesnakes responded to timber harvesting. Mortality associated with implantation of radio transmitters has been recognised in snakes during conservation studies. Late summer implantation prior to entering a hibernacula has been demonstrated as one of the most significant causes of mortality (RUDOLPH *et al.*, 1998) with a higher risk in smaller snakes. Snakes should not be implanted during the late season when mortality can approach 100 %. Concerns include radio transmitter morbidity exceeding 66 % related to infections and inflammation (LENTINI *et al.*, 2011). This potentially raises concerns with behaviour data validity related to decreased or increased movement, basking length and frequency. An Association of Zoo and Aquariums task force (AZA 2013) has expanded specific guidelines for species like the Eastern Massasauga Rattlesnake (*Sistrurus catenatus catenatus*) advising to not place radio transmitters in snakes under 200 grams, limit transmitter size to less than 50 % of snake width and 2.5 % of weight in gravid females compared to 5 % maximum in males. This also advises to avoid implantation after June in gravid females of this species and after mid-August completely. Expanding specific surgical guidelines to other species can help towards a conservation research goal of documenting post hibernacula survival rates the following spring and lessening any impact on reproduction.

Many small transmitters only last a season, so many reports and papers lack any follow up on the snakes' health the following spring. Morbidity and mortality are not routinely published as data in the herpetology summaries. Almost 9.5 % acute case fatality was reported from anaesthesia and post-surgical complications in a small northern population of Lake Erie Watersnakes (*Nerodia sipedon insularum*) (KING *et al.*, 2003) The surgical protocol maintained snakes in captivity for three days prior to release. Debate exists among herpetologists considering time in captivity an additional and unnecessary stressor and favoring immediate release upon recovery (UJVARI and KORSOS, 2000). One task force (AZA 2013) now stipulates that snakes should be kept at least 24 hours after surgery for observation and advises 3-6 days depending on the need for antibiotics considering environmental factors during release.

## Materials and methods

Forty-seven snakes had 71 implantation surgeries. A second surgery was done two to three years later on 22 snakes to remove and replace the transmitter prior to battery loss and two of these snakes had a third removal and transmitter replacement. Transmitters weighed 16 or 28 grams and had a two or three year battery life. Transmitter size was chosen so as not to exceed 5 % of the snake's body mass. Initial control of each snake was made safer utilizing the Pro Bagger system and clear plastic restraining tubes (Midwest Tongs, Inc, Greenwood, MO USA). Snakes were then safely manually restrained with their head inside the tube. Isoflurane gas (IsoFlo. Abbott Laboratories, Chicago, IL, USA) was connected at the opposite end using the tube for mask induction and maintenance anaesthesia with Doppler monitor for heart rate. Regional nerve blocks pre-surgically with mepivacaine HCl at 2 - 6 mg/kg (Carbocaine-V. 20 mg/ml. Pfizer. Pharmacia & Upjohn Company, New York, NY, USA) were placed dorsally on three ribs, at the anticipated surgical site. Incision between the first and second rows of lateral scales on the left, typically 22 cm cranial to the cloaca to account for the transmitter antennae caudally. Routine coelomic implantation of the AI-2 Holohil transmitter caudal to the lung necessitated an incision only the transmitter width and included use of a polypropylene urinary catheter as a stylet for subcutaneous placement of the transmitter wire distally. Two-layer closure was accomplished with poliglecaprone 25 with triclosan suture (Monocryl Plus, Ethicon, Inc. Johnson & Johnson. West Somerville, NJ, USA) with the deep muscle layer including one suture around the antennae wire as an anchor. Topical tissue adhesive (GLUture. Abbott Animal Health. Chicago, IL, USA) was used after suturing the skin at the implantation site, near the cloaca where the antennae stylet was removed and after microchip placement between ventral scutes. Enrofloxacin at 5 - 10 mg/kg IM q 24hrs x 3 doses (Baytril 22.7 mg/ml. Bayer Healthcare LLC, Shawnee Mission, KS, USA) and injectable meloxicam at 0.2 mg/kg IM q 24 hrs x 3 doses (Metacam 5 mg/ml. Boehringer Ingelheim. Fort Dodge, IA USA) post-surgically were provided while the snakes were maintained in captivity the initial 48 hours (THOMPSON *et al.*, 2014) (HALE *et al.*, 2014).

## Results and discussion

No acute mortality was noted in 47 Timber Rattlesnakes (*Crotalus horridus*) with 71 coelomic surgeries and only a single overwintering mortality observed in a large adult male. (THOMPSON *et al.*, 2014) GIS movement data for 22 snakes that received a replacement transmitter were retrospectively compared one and two weeks pre- and post- surgery and found no statistical difference (HALE *et al.*, 2017). Two post- surgical infections were observed in the field. The first included transmitter removal and one month of topical and systemic wound therapy for an enrofloxacin resistant strain of *Streptococcus* (THOMPSON *et al.*, 2014) The second did not have any treatment since the removal and

post treatment wound management would have crossed into hibernacula timing. Both snakes were identified alive outside their hibernacula the following spring. Transmitter inflammation post-surgically could increase basking times and thus predation or ophidiophobic human induced mortality. Further studies are warranted.

These authors seek to increase dialogue on improving survival and limiting morbidity associated with Snake Transmitter Diseases (STDs). Concerns with a 19 % Holohil transmitter failure rate (KING *et al.*, 2003) were not observed by these authors with the larger 16 and 28 gm transmitters possessing a 2-3 year battery life, allowing multiple years of tracking. Veterinarians with a conservation focus are encouraged to seek out collaborations and dialogue with herpetologists to improve compliance with IACUC welfare goals regarding surgical technique, asepsis and pain management. This will hopefully lead to reductions in morbidity and mortality and improvement in the conservation data gathered and reported in reptile species being studied.

## Acknowledgements

This paper is a contribution of the Hardwood Ecosystem Experiment. We gratefully acknowledge veterinarians Tomohito Inoue, Jeff Ko, Ray Wack, and Nancy Anderson for providing guidance on this project. We also thank biologist Brian MacGowan and herpetologists Jami MacNeil, Sarabeth Klueh-Mundy, and Zack Walker for their fieldwork and pre-/postsurgery assistance, which made this research possible. Funding was provided by the Indiana Division of Fish & Wildlife, Wildlife Diversity Section (State Wildlife Improvement Grant T07R08); the Indiana Division of Forestry, and Purdue Department of Forestry & Natural Resources. Initial abstract oral presentation at ExoticsCon 2016. Portland, Oregon, USA

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## TAKE A WALK ON THE WILD SIDE – HOW CAN ZOO-BASED VETERINARIANS “HELP” WILDLIFE?

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### Summary

Today practically all species live in dynamic multi-use landscapes in which anthropogenic activities have become the main driver of environmental and social change. It is within this context that I discuss the opportunities, challenges and limitations that accredited zoo-based veterinarians face when working with free-ranging wildlife. Adopting a modern wildlife health concept beyond parasites and pathogens incorporating social and environmental factors appears essential. It is equally clear that solving problems that impact the health of wildlife and biodiversity conservation necessitates participatory approaches that bridge traditional disciplinary silos

### Introduction

Today practically all species live in dynamic multi-use landscapes in which anthropogenic activities have become the main driver of environmental and social change. We have clearly left the unusually stable 10,000 years of the Holocene behind us and have firmly entered into the Anthropocene (RÖCKSTRÖM *et al.*, 2009). Due largely to the reliance on fossil fuels, the expansion of industrial agriculture and land transformation in a society reliant on constant growth, the Earth's regulatory mechanisms, that have in the past provided consistent temperatures, freshwater availability and guaranteed biogeochemical flows are being severely perturbed. Humankind is potentially, irreversibly, leaving the safe operating space for human existence on earth (STEFFEN *et al.*, 2015). Additionally, global socio-political turmoil with starkly increasing economic inequality, polarised value-systems, rejection of science and subsequently the disregard of evidence-based policy decisions, asymmetrically shifts cost-benefit ratios away from sustainable environmental and biodiversity conservation towards further unbridled economic development.

It is within this context that I discuss the opportunities, challenges and limitations that accredited zoo-based veterinarians face when striving to work with free-ranging wildlife. Firstly it is important to note that this discussion is not new but has been consistently lead over the past two decades. The role of zoo-based veterinarians in wildlife health and conservation was firmly established amongst others at the Bronx Zoo and subsequently clearly recognised *inter alia* in the initial and most recent World Association of Zoos and Aquariums (WAZA) conservation strategies (WAZA, 2005; BARONGI *et al.*, 2015). Somewhat surprisingly it appears that discussions are still warranted today.

As today's scientific communities consistently strive forwards in ever-narrowing clearly delineated and protectionist speciality fields, it appears prudent to pause on the perceived dichotomy of zoo-based and free-ranging wildlife veterinarians as reflected in our respective organisations and specialisation degrees. There is only ever one “wildlife” and this holds true irrespective of holding conditions and context. While in the past living conditions were seen as vastly different between free-ranging and zoo-housed individuals this delineation has become more pervasive in the past decade. As anthropogenic encroachment continues, free-ranging wildlife subsists on ever-smaller islands, often

frighteningly similar to zoos, in an ocean of human development. Today all wildlife survives along a gradient of increasing human encroachment and disturbance and subsequently, wildlife veterinarians no longer work in distinct entities but somewhere along this same gradient (figure 1).

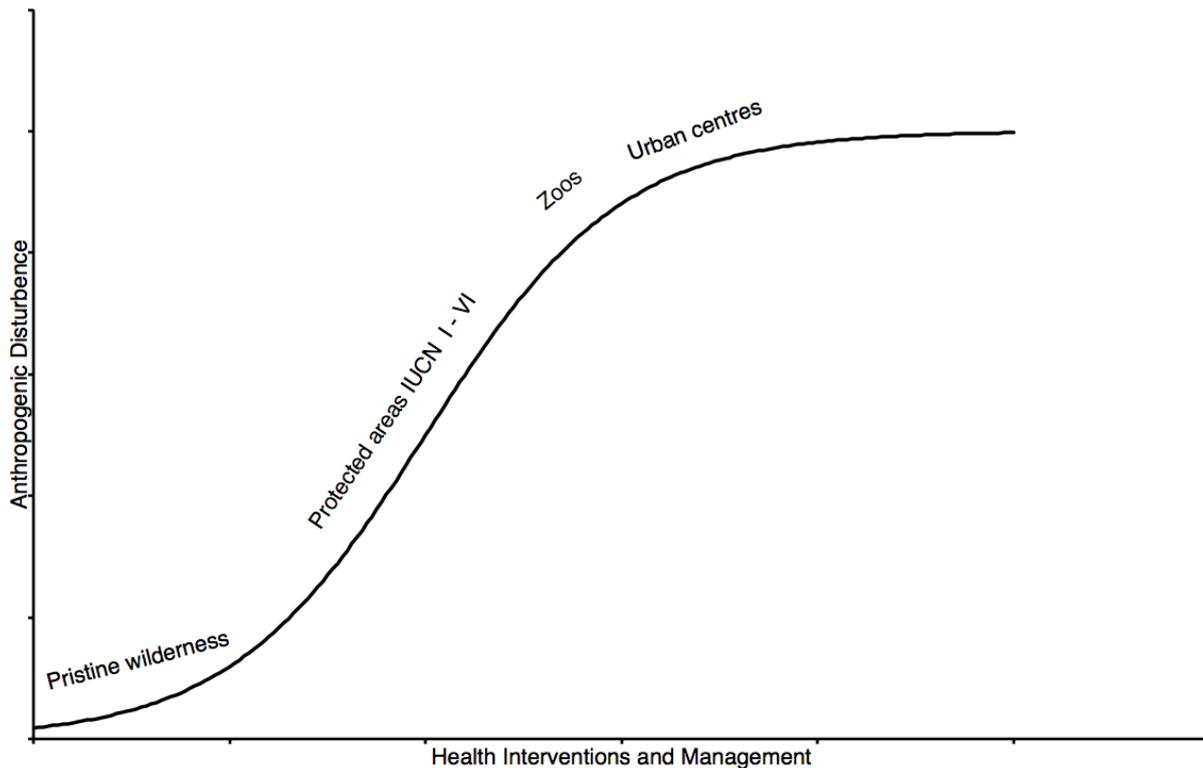


Fig. 1: Wildlife survives along a gradient of increasing human encroachment and disturbance with similarly increasing health interventions and management

Not only has our environment changed in the past decades but also our understanding of health and disease. Unfortunately, wildlife health along the proposed gradient appears in many instances entrenched and focused on disease (STEPHEN, 2014). The largely anthropocentric and human medical-driven One Health concept has in the past two decades exacerbated this situation by primarily highlighting wildlife as a source of infections and threats to public health while neglecting the value of biodiversity and the associated services (STEPHEN, 2014; DEEM, 2015). Today consensus exists that wildlife health, like human health, must be viewed beyond parasites and pathogens, incorporating social and environmental factors while considering individual attributes and behaviours (STEPHEN, 2014).

## Results and discussion

In order for accredited-zoo-based veterinarians to fully participate and lead in the field of wildlife health they necessarily must embrace a dynamic holistic approach to health and be fully aware of, and understand the pressing present-day conservation challenges (DEEM, 2015). Wildlife health, the capacity to cope with change, results from complex interactions of biologic, environmental, and socio-economic factors (STEPHEN, 2014). Clearly a modern and holistic approach to wildlife health necessarily integrates across previously distinct disciplines such as physiology, ecology, animal behaviour, conservation biology and many more.

Beyond adopting a modern wildlife health concept and cognizant of the fact that health definitions and actions may be time and space constrained it appears essential that wildlife veterinarians along the gradient reach consensus on a common goal. If the goal is to “help” wildlife then maintaining resilient individuals, populations and landscapes along the gradient is paramount. Here the transdisciplinary, ecology-driven and conservation-centred, conservation medicine approach can provide guidance (TABOR, 2002; DEEM, 2015).

A possible constraint for zoo-based veterinarians can arise from divergent goals at the institutions they are employed at. Some 500 institutions are accredited in North America (AZA) and Europe (EAZA). While these institutions have all signed-on to further conservation in the accreditation process there are certainly varying degrees of commitment and understanding in respect to conservation activities and more specifically *in-situ* conservation measures. Similar to the wildlife veterinarians as a profession, accredited institutions need to reach a consensus as to where and how far their responsibilities reach on the gradient.

In this challenging environment, seemingly novel cross-disciplinary approaches to animal, human and environmental health have been developed, defined and branded. Conservation Medicine and One Health are but two of the concepts that are being widely discussed and equally hyped. While intensive work within disciplines is essential when developing expertise, it is equally clear that solving problems that impact the health of wildlife and biodiversity conservation necessitates participatory approaches that bridge the traditional disciplinary silos (WALZER, 2016).

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## VETERINARY INPUT INTO THE ZOOLOGICAL SOCIETY OF LONDON (ZSL) AMPHIBIAN CONSERVATION PROGRAMMES

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The veterinary teams in the Zoological Society of London (ZSL) Living Collections and Institute of Zoology work closely with ZSL's herpetology team to promote health and welfare in ZSL's amphibian conservation programmes. The Living Collections' team has developed quarantine and health screening protocols for amphibian species. Best husbandry practices have been researched, including UV-B radiation requirements for the Critically Endangered mountain chicken (*Leptodactylus fallax*) and substrate preference in the caecilian *Geotrypetes seraphini*. Disease surveillance has led to detection of novel diseases such as intestinal adenocarcinoma in *L. fallax* and chytrid fungus (*Batrachochytrium dendrobatidis* or Bd) infection in a wild neotropical caecilian. Novel treatments have been developed, including the use of itraconazole for treatment of Bd infection in captive caecilians. Wildlife veterinarians in ZSL's Institute of Zoology have played a key role in understanding the emergence, spread, impact and mitigation of amphibian infectious diseases world-wide, including identification of ranavirus infection as a cause of wild amphibian mortality in Europe, discovery of Bd as a cause of global amphibian population declines, mitigation of amphibian chytridiomycosis and the role of the amphibian trade in the spread of Bd and *B. salamandrivorans*. A citizen-science disease surveillance programme for native amphibian species has been established and advice documents produced for the public regarding amphibian disease. Methods of disease risk analysis for interventions for conservation purposes have been developed, including disease risk analysis and post-release health surveillance of the reintroduced pool frog (*Pelophylax lessonae*) in the UK.

## WHY DO ANIMALS IN THE ZOO DIE?

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The most common cause of death in zoo animals around the world remains unclear as not many extensive reports or reviews are published on this topic. Several international databases developed for exotic and wild animals collect veterinary records but usually with limited success depending on complexity of veterinary services and willingness to share sensitive information. The huge variety of different animal species makes answering this question extremely difficult. This information could greatly increase clinical monitoring and improve preventive veterinary health care in zoological gardens, even though death etiology is probably different in different zoos and depends on many factors. Although this is only a brief summary, a retrospective overview of the causes of death of animals from the zoo Ljubljana in the years 2005 – 2015 can suggest new possibilities to develop the zoological veterinary service. During this 10 year period, a total of 330 animals were submitted for necropsy, of which 225 were mammals, 80 birds and 25 reptiles. The most common cause of death was due to septicemia (20 %), infectious diseases (12 %), trauma (11 %), neoplasias (5 %), parasitosis (4 %), intoxications (4 %), reproductive diseases (3 %), abortion (3 %), kidney diseases (3 %), liver diseases (2 %), and rare cases of cardiopathies, mycotoxicosis, death due to old age, incarcerated hernias, amongst others. The results of this retrospective study bring a general overview of the epizootiological situation in the zoo Ljubljana and also valuable information to other zoos to optimize preventative plans and diagnostics.

## HOW CAN AN ANIMAL POISON CALL CENTRE BE INVOLVED IN VULTURES CONSERVATION?

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### Summary

You do not need to be a wildlife vet running through the jungle to contribute to wildlife preservation. The National Centre for Veterinary Toxicologic Information (CNITV), located in Lyon (France), is an original key player in vulture conservation in France. Currently collaborating with other french nature conservation associations for reintroduction of the Bearded Vulture (*Gypaetus barbatus*), within the scope of the LIFE GYPCONNECT (LIFE14 NAT/FR/000050), the part of CNITV is about mortality monitoring, through necropsies of dead vultures collected within the action perimeter of the program, and toxicological analysis, to determine current threats for reintroduced birds. To date, 25 necropsies have been performed. Main death cause, anthropogenic, is electrocution (9/25), followed by trauma (7/25), and infectious diseases (6/25). Quite high levels of lead (> 6 ppm/MS) have been detected in some cases (3/25), showing an exposure to this toxic element, and raising the question of lead sources in these areas, and of the potential threat of the use of lead ammunitions. This case of field data collection exposes once again the essential part of vets in monitoring reintroduction programmes, and is quite a unique example of the implication of a poison call-centre in this monitoring.

### Introduction

The National Centre for Veterinary Toxicologic Information (CNITV), located in Lyon (France), is a non-profit organisation created in 1976, to answer 24/7 to any question about domestic or wild animals poisoning, helping for diagnosis and treatment. Over time its missions have evolved and extended, and in 2003 CNITV started to take a part in LIFE's, European programmes promoting biodiversity. After contributing to the preservation of the Egyptian Vulture (*Neophron percnopterus*) and of the Lesser Kestrel (*Falco naumanni*), the centre is now working with the LPO (Ligue de Protection des Oiseaux) and other french nature conservation associations, within the european LIFE project GYPCONNECT (LIFE14 NAT/FR/000050), for reintroduction and protection of the Bearded Vulture (*Gypaetus barbatus*). Global population of this scavenger bird, from Accipitridae family, feeding mainly on bones from carrion, is currently declining. In Europe, the population was estimated to 1,200-1,600 mature individuals in 2015 (BIRDLIFE, 2017). Therefore LIFE GYPCONNECT, a 6-year-long programme started in 2015, is working on reintroducing captive-born birds in Massif Central and Pre-Alps, to re-create an ecologic corridor between this new population and the endemic one in the Pyrenean mountains, and then allowing the emergence of a metapopulation from Spain to the Balkans.

Within LIFE GYPCONNECT, CNITV is mainly in charge of toxicologic monitoring, from organisation to realisation, especially for collecting, consolidating and checking traceability of vultures mortality data in the action perimeter of the program. Other missions of CNITV, in association with other LIFE GYPCONNECT operators, are communication about GYPCONNECT, scientific diffusion and

administrative and financial tasks. Here are the first results of LIFE toxicologic monitoring, as an intend to draw some highlights about main threats for bearded vultures in the reintroduction area.

## Material and methods

First step of CNITV action in the programme was to provide a training course about how to collect and preserve properly vultures corpses, for all operators susceptible to encounter some, in order to provide as many data as possible for each necropsy, and to ensure good sanitary conditions. For every vulture collected, a data sheet was added, specifying identity of the bird, other affected/dead birds on site, traces of dubious products around (baits, wrapping), precise location, environment features (power lines, feeding spot, houses), weather conditions, others noteworthy facts in the perimeter.

The collection area extends from Aude department, close to eastern Pyrenean mountains, to regional park of Baronnies (Drôme) and Vercors (Drôme and Isère), creating an approximated curve from eastern Spanish to southern Italian borders, and all species of birds of prey founded dead are collected for necropsy. To date, 25 necropsies have been performed by CNITV vets. For every carcass, a necropsy report is registered in an in-house database, to allow further exploitation of data collection. Every report provides accurate descriptions of lesions, and pictures. Analysis are performed for every dead vulture, on liver samples, by La Drôme Laboratoire (LDA 26, Valence, Drôme), for lead and other metals levels analysis, and Ecoloxie-La Voulte (La Voulte-sur-Rhône, Ardèche) for toxicological screening. A liver sample is also send to research unit USC1233 Rongeurs Sauvages (VetAgro-Sup, Marcy l'Etoile, France) for Vitamin-K antagonists (VKA) screening.

## Results and discussion

### Species collected - Location of death

In total, 19/25 vultures were Griffon vultures (*Gyps fulvus*), 2/25 Cinereous vultures (*Aegypius monachus*), 2/25 Golden eagles (*Aquila chrysaetos*), 1/25 Common buzzard (*Buteo buteo*), and 1/25 Bearded vulture (*Gypaetus barbatus*). This significant majority of Griffon vultures is easily explained by the predominance of this species in the programme area: as an example, the last census of LPO Grands Causses, in 2010, counted 283 breeding pairs of Griffon vultures vs about 20 pairs of Cinereous vultures (data from LPO Grands Causses website).

Five geographic areas have been defined, each related to one association or park organisation taking care of carcasses collection in this area: Aude, Grands Causses, Hérault, Vercors, Baronnies. In this first slack of data, Grands Causses and Baronnies areas are more represented (respectively 10/25 and 9/25). To date no environmental feature related to these results was highlighted. Furthermore, this may be linked to operator bias, as LPO Grands Causses and "Vautours en Baronnies" association are on field collecting carcasses on a more regular basis.

### Causes of death

Main cause, anthropogenic, is electrocution (9/25), followed by trauma (7/25), and infectious diseases (6/25), matching data in literature about threats for declining vultures populations (FERGUSON-LEES AND CHRISTIE, 2001). To reduce electrocution occurrence, LIFE GYPCONNECT is working with ENEDIS, french company in charge of the electrical network, to identify risky areas and to secure lines. A veterinary thesis is ongoing to make a synthesis of mortality causes.

Quite high levels of lead (> 6 ppm/MS) (KENDALL *et al.*, 1996) have been detected in some cases (3/25), showing an exposure to this toxic element. 2 other levels (respectively 3.65 and 3.73 ppm/MS) are dubious.

Those high levels are raising the question of lead sources in these areas: is there a potential threat of the use of lead ammunitions, as described in literature? (FERGUSON-LEES AND CHRISTIE, 2001)

As lead is a well-known threat, another purpose of the LIFE program is to test the use of lead-free ammunitions, as it was done in the US to protect reintroduced Californian condors (*Gymnogyps californianus*) (GREEN *et al.*, 2008). A constant toxicological monitoring is necessary to assess the effects of this lead ban, enhancing the part of CNITV in the reintroduction process.

Moreover, 2/3 high levels and 1/2 dubious were measured in carcasses found in the same area (Grands Causses): even if there are too few results to firmly draw any conclusion, it could let us think about some illegal deposits of hunting remains and offal. This draws attention about the necessity to collaborate with hunters to find the best solutions to prevent lead ingestion by scavengers.

### VKA exposure

Vitamin-K Antagonists (VKA) exposure from field treatment with rodenticides has been reported in literature, as there were several confirmed cases of anticoagulant rodenticide sub-lethal exposure in Red Kites (*Milvus milvus*) in Auvergne, a french region located near the LIFE area. (COEURDASSIER *et al.*, 2014) From this data, it was decided to quantify VKA exposure for vultures in the LIFE area, to determine if rodenticide treatments could be a threat for bearded vultures reintroduction. This study will be the subject of a veterinary thesis, but to date 16 VKA-screenings were performed by Dr Isabelle Fourel from USC1233. 5/16 were positive, with total levels of anticoagulant rodenticides (AR) ranging from 3.7 to 54.2 ng/g wet weight (wet wt.), and 4/5 positive samples contained 2 or more different AR. Most represented were difethialone and bromadiolone (both present in 4/5 positive samples). These levels aren't lethal (no VKA lesions at necropsy), but they clearly highlight an environmental exposure to anticoagulant rodenticides: further investigations would be necessary to find the source of this exposure, for example analysis of liver samples from dead wild boars and other animals from bearded vultures' diet, which could have ingested AR baits.

### Acknowledgements

We would like to thank all LIFE operators working on field to collect dead vultures, and more widely all participants contributing to make LIFE GYPCONNECT possible. We also want to thank the USC1233 Rongeurs Sauvages, and especially Dr Isabelle Fourel, for her collaboration in VKA study.

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**BANK ON IT! THE EAZA BIOBANK**

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The EAZA membership has recently established dedicated biobanking facilities for the European zoo community. This EAZA Biobank aims to be a primary resource for supporting population management and conservation research, and will be an invaluable resource for zoo and wildlife veterinarians. The EAZA Biobank aims to hold blood/ DNA/ tissue and serum from all animals in European zoos, and be designed such that samples are registered in ZIMS and are available to benefit efforts to ensure the genetic and physical health of intensively managed populations. Ideally, every animal is sampled at least once during routine veterinary practices for the purpose of blood biobanking. Currently only a very small fraction of animals are sampled upon death (< 10 %) and efforts should be taken to increase this number, as they will be valuable sources of information for the management of the extant populations. The biological samples will enable DNA-analysis which will be a key tool to increase the European Ex-situ Programmes' chances of success, by improving the knowledge of relatedness, resolve paternity issues, identifying the origin of individuals to help set up the correct breeding groups and ensure that, as far as possible, captive populations represent the genetic diversity of their wild counterparts. The unique coupling of whole blood and serum samples will enable medical/ epidemiological research of interest for veterinarians and enable e.g. disease surveillance of infectious diseases at small- as well as meta-population level, enable retrospective surveys, veterinary molecular diagnostics and identifying genetically inherited diseases.

## A MULTI-DISCIPLINARY APPROACH TO THE CONSERVATION OF GYPS VULTURES IN SOUTH ASIA – A ROLE FOR VETERINARY PROFESSIONALS

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### Summary

Since the 1990s *Gyps* vultures resident to South Asia have undergone unprecedented declines and become Critically Endangered. Multi-disciplinary research, conservation breeding and advocacy work have likely saved these species from extinction. Veterinary professionals working in a number of guises have contributed to this remarkable conservation success. Next steps must be to attempt a degree of re-population; through continued efforts to make and keep the environment safe, by breeding more birds for release, and through monitoring the success of these birds post-release.

### Introduction

In less than two decades, and with very limited resources, an international consortium of organisations and individuals from multiple disciplines, have collaborated to effectively save *Gyps* vultures resident to South Asia from extinction. Such success for an 'in-country' programme is to be greatly commended, and bears testament to the dedication of local staff as much as that of regional and international biologists. A regional Recovery Plan developed in 2004 ([www.save-vultures.org/resources/action-plans](http://www.save-vultures.org/resources/action-plans)) recommended three main actions: removal of diclofenac from veterinary practice, the identification of safe alternative drugs, and the immediate establishment of a conservation breeding programme. This plan has been updated annually as 'A Blueprint for the Recovery of South Asian *Gyps* vultures' (SAVE, 2016). Highlights include the establishment of natural and artificial breeding under challenging conditions, and the increased reproductive rate of all three species as a result. Veterinary support has been constant and varied, and an integral component of the programme throughout; ensuring healthy populations for future releases.

### Material and methods

#### Species

One of the authors (VP) first identified declines and mortality of oriental white-rumped vultures (*Gyps bengalensis*) (WRV) in Kaoladeo National Park, Rajasthan (PRAKASH 1999). This led to the Bombay Natural History Society (BNHS) calling upon the Royal Society for the Protection of Birds (RSPB), and

in collaboration, a local investigation of the issue by a veterinarian from the Zoological Society of London (ZSL) realising that the problem extended beyond the national park. Unprecedented national declines of the WRV and the long-billed vulture (*G. indicus*) (LBV) were subsequently identified and quantified (PRAKASH *et al.*, 2003). Conservation efforts since have focussed on these and the closely related slender-billed vulture (*G. tenuirostris*) (SBV), also shown to have undergone a precipitous population decline in India (PRAKASH *et al.*, 2007). All three species are resident to South Asia and all now classified as Critically Endangered ([www.iucnredlist.org](http://www.iucnredlist.org)).

### Research

Veterinary professionals have been involved in all manner of multi-disciplinary research relating to the conservation of these species, including the early investigation of declines. Carcasses and the tissues of dead vultures were examined to establish cause of death where possible (CUNNINGHAM *et al.*, 2003, OAKS *et al.*, 2004a, SHULTZ *et al.*, 2004), and modelling employed to establish whether such declines could be fully attributed to the proposed cause elucidated (GREEN *et al.*, 2004). A multi-zoo metanalysis quantified the risk of commonly-used non-steroidal anti-inflammatory drug (NSAID) therapy across avian taxa (CUTHBERT *et al.*, 2007). Subsequently, multiple studies have established the relative toxicity of NSAIDs in *Gyps* vultures (SWAN *et al.*, 2006, SWARUP *et al.*, 2007, NAIDOO *et al.*, 2009, SHARMA, 2012, SHARMA *et al.*, 2014, FOURIE *et al.*, 2015), and in some instances in other raptors and wider avian taxa. Overt and covert surveys of pharmacies selling veterinary and human drugs in the region were conducted from 2007 to 2015 to establish the availability of various NSAIDs, trends in their use, and general enforcement of any legislation relating to these drugs (CUTHBERT *et al.*, 2011, GALLIGAN, unpublished data). Tissue sampling of the carcasses of dead cattle, and subsequent liquid chromatography-mass spectrometry of these samples, established the residue prevalence of various NSAIDs in fallen livestock available to vultures in the region over time (TAGGART *et al.*, 2009).

### Conservation breeding

The rate of decline of wild vultures indicated that conservation breeding was required to prevent extinction at the national or global level. Multiple organisations have collaborated to maintain healthy captive breeding populations of all three worst-affected species; to ensure a lack of exposure to NSAIDs and increase numbers to supply healthy young birds for release, once the environment is deemed adequately safe. Veterinary input has been an important component of the programme. Birds were mostly sourced from rescue centres and as nestlings from remaining nesting sites. Temporary quarantine facilities were built away from vulture conservation breeding centres (VCBCs), and protocols to safely populate these centres with healthy birds established (PRAKASH *et al.*, 2012). VCBCs were designed and built to accommodate sufficient numbers of birds in a near-natural environment to maintain adequate health, welfare and breeding. Experiences from similar projects (Eurasian griffon vultures (*Gyps fulvus*) in Europe and California condors (*Gymnogyps californianus*) in the USA) were sought to inform these designs and management guidelines (PRAKASH *et al.*, 2012). A preventive medicine programme was designed to include; annual health checks of all birds – physical examination, blood collection for haematological (and where appropriate biochemical) analysis, faecal examination for pathogenic parasites (and where appropriate bacteria); the reactive examination of any individuals where health status was a concern; and examination at *post mortem* of all birds that died and any eggs that failed to hatch (where accessible), all to ensure adequate disease surveillance (NOLLET AND CALVI, 2010). Organisations involved in the management of the VCBCs in range countries employed veterinarians to oversee the health and welfare of birds, and regular visits from veterinarians associated with the programme through partner organisations were used as opportunities for training and knowledge exchange. Due to a lack of sexual dimorphism, sex

identification of birds through molecular techniques was employed on captive birds to optimise sex ratios within colony aviaries and maximise natural breeding success (GHORPADE *et al.*, 2012).

### **Release plans**

Assessment of the risk to released birds must be established prior to releases. Vulture Safe Zones (VSZs) are proposed as a means to provide a limited safe environment. Only healthy birds are likely to survive and establish self-sustaining free-living populations, so pre-release health checks will be designed to ensure this. Success will only be measurable through post-release health monitoring. For such wide-ranging species the only practical method of tracking and finding individual birds (especially important in the case of morbidity or mortality), is satellite telemetry; suitable systems must be attached to all released birds and remain functional without compromising their health or welfare. A representative sample of captive birds was fitted with 'dummy' satellite tags in order to assess this.

### **Advocacy**

Engaging the support of all stakeholders – including national and state governments, national park authorities and staff, conservation NGOs, veterinary professionals, farmers, pharmaceutical companies and general public – was seen as critically important to the likelihood of success; once established, measures to remove the cause of the declines would necessarily involve all these groups.

## **Results and discussion**

### **Species**

Road transect surveys of the three resident *Gyps* vulture species were undertaken in 1992, 2000, 2002, 2003 and 2007. Declines in all three species were shown to exceed 96 % between 1992 and 2007 across western, northern, central and north-eastern India (PRAKASH *et al.*, 2007).

### **Research**

Initial pathological investigations identified either infectious disease or poisoning as the most likely cause of death driving the population declines (CUNNINGHAM *et al.*, 2003). Although infectious agents were found (e.g. mycoplasma and herpesvirus, OAKS *et al.*, 2004b, CARDOSO *et al.*, 2005), inadvertent fatal poisoning by an NSAID, diclofenac, was identified as driving the declines (OAKS *et al.*, 2004, GREEN *et al.*, 2004, SHULTZ *et al.*, 2004). A survey conducted by questionnaire sent to zoological collections, wildlife centres and avian veterinarians worldwide, analysed the treatment outcomes from more than 870 birds of 79 species administered NSAIDs. Diclofenac, carprofen, flunixin, ibuprofen and phenylbutazone were all associated with mortality to varying degrees. No mortalities occurred following treatment with meloxicam, used in over 700 birds from 60 species (CUTHBERT *et al.*, 2007). This study demonstrates the importance of veterinarians working in zoological collections maintaining and sharing records of failures as well as successes. NSAID studies on captive *Gyps* vultures have subsequently shown meloxicam to be non-toxic at all doses of inadvertent likely exposure in nature (SWARUP *et al.*, 2007), whilst multiple studies investigating several other NSAIDs (diclofenac, ketoprofen, flunixin, carprofen, phenylbutazone and aceclofenac) have demonstrated toxicity to *Gyps* vultures (SWAN *et al.*, 2006, NAIDOO *et al.*, 2010, SHARMA, 2012, FOURIE *et al.*, 2015) and other scavenging birds (SHARMA *et al.*, 2014). Pharmacy surveys show that the relative use of diclofenac since 2007 has reduced whilst that of meloxicam has increased (GALLIGAN, unpublished data). Residues of diclofenac, and eight other NSAIDs known to be used by veterinarians in the Indian sub-continent, were evaluated in almost 1500 liver samples taken from fallen domestic livestock from across seven Indian states. Diclofenac was present in more than 11 % of samples, and other NSAIDs

at lower residue prevalence (TAGGART *et al.*, 2009). Such prevalence is not compatible with recovery of resident *Gyps* vulture populations, but subsequent surveys employing the same methods, show significant reduction in residues. Studies suggest a residue prevalence of less than 1 % of carcasses is necessary for future recovery and Vulture Safe Zones should therefore aspire to this.

### Conservation breeding

A growing conservation consortium, now comprising 20 partners and multiple associates, has developed over the intervening years, and was first formalised as SAVE (Saving Asia's Vultures from Extinction) in 2011. ZSL has provided veterinary input at the three core VCBCs in India, and one in Nepal, throughout this time. Husbandry guidelines were developed (PRAKASH *et al.*, 2012) as was a Veterinary Care Manual (NOLLET AND CALVI, 2010). Where funding allowed, VCBCs were built in isolation of established zoos in order to prevent contact with non-native birds and pathogens, thus facilitating eventual release into the wild. Annual health checks were undertaken in every bird until 2015, at which point these were targeted to all birds hatched that year, any birds demonstrating health concerns, and a further 10 % plus one of the birds in each aviary (as a representative sample). Haematological norms have been established for each species (unpublished data). As well as assessing captive population health through these checks, opportunities to mentor local staff in the routine care of these birds in captivity were taken. Low levels of parasitism have been permitted as certain parasites, such as lice and cestodes, might be species-specific and as endangered as their hosts. Also, the long-term health of released birds might be jeopardised if reared in the absence of natural parasites. Blood samples harvested at these annual health checks were used to provide DNA for molecular sexing, undertaken at VCBC Pinjore (for birds in India). Samples from birds in Nepal could not be exported, so expertise developed at VCBC Pinjore was employed in Nepal to allow molecular sexing from similar samples at the Agriculture and Forestry University in Chitwan. Sex identification allowed re-assortment of birds in Nepal in 2016 and subsequently nine chicks have hatched in 2017. At VCBC Pinjore, first successful hatchings took place on the nest in 2007/8. Since then a total of 163 nestlings have hatched and survived; 40 on the nest and 123 through artificial incubation and double-clutching. By the end of 2016 the four centres across northern India and Nepal (plus a newly built VCBC in Bhopal – populated with birds from VCBC Pinjore – and two other VCBCs at Hyderabad and Junagarh) housed a total of 544 *Gyps* vultures and during the 2015/2016 breeding season produced a total of 58 nestlings (representing all three species); 39 in total at Pinjore (9 WRV, 26 LBV and 4 SBV). Incidence of serious disease and mortality has been low; a review of records at VCBC Pinjore showed that between 2005 and 2017 a total of 23 birds died (see Table 1). Likely cause of death was established in only 12 cases, but was sporadic and varied. Only one 'disease event' caused multiple deaths; an attack by bees. Across all centres, during the course of 2015, a total of 4 birds died (< 1 % of the overall population). All these deaths were of adults – three WRVs and one SBV – one with chronic health issues (arthritis), one with probable septicaemia, and two of undetermined cause. During the 2011/2 breeding season some hand-reared juveniles developed subtle signs of metabolic bone disease (bowing of long-bones) and a protocol was established to prevent any future incidence; chicks to receive at least 15 minutes of direct natural sunlight twice daily from day three, in addition to calcium supplementation calculated according to (rapidly changing) body weight and food intake.

Table 1: A review of mortality records at VCBC Pinjore between 2005 and 2017.

Species	Ring	Death date	Age, Sex	Cause
<i>Gyps bengalensis</i>	A19	04-01-05	Adult, Male	Bee attack, died same day as honey buzzard disturbed nest and caused swarm to fly through aviary
<i>Gyps bengalensis</i>	A17	05-01-05	Adult, Male	Bee attack
<i>Gyps bengalensis</i>	A11	05-01-05	Adult, Male	Bee attack, neck swollen with stings, died next day despite supportive treatment
<i>Gyps bengalensis</i>	A10	01-02-05	Adult, Male	Bee attack, died later despite supportive treatment, dehydrated and renal failure?
<i>Gyps indicus</i>	B26	25-06-05	Juvenile, Unknown	Cause unknown
<i>Gyps bengalensis</i>	A36	28-12-06	Adult, Unknown	Chronic haemorrhagic anaemia due to endo and ecto parasites
<i>Gyps bengalensis</i>	A06	21-02-07	Adult, Unknown	Cause unknown
<i>Gyps indicus</i>	B40	11-11-07	Adult, Unknown	Cause unknown
<i>Gyps bengalensis</i>	A49	26-02-08	Adult, Male	Cause unknown
<i>Gyps bengalensis</i>	A38	02-05-08	Adult, Male	Cause unknown
<i>Gyps bengalensis</i>	B93	26-06-08	Adult, Unknown	Thin and dehydrated for > 1 week. Died despite treatment. Splenomegaly and foreign body in liver - but PM report incomplete
<i>Gyps bengalensis</i>	A24	16-09-10	Adult, Male	Trapped as adult from wild - likely old bird. Three months depression and anorexia. Emaciated. Abscess/neoplasia of lung? Airsacculitis
<i>Gyps bengalensis</i>	A25	05-02-11	Adult, Male	Cause unknown
<i>Gyps bengalensis</i>	B95	23-06-11	Adult, Male	Cause unknown
<i>Gyps bengalensis</i>	A46	11-07-11	Adult, Male	Cause unknown
<i>Gyps bengalensis</i>	A12	24-08-11	Adult, Female	Cause unknown
<i>Gyps bengalensis</i>	A32	13-10-11	Adult, Male	Died due to bumblefoot infection
<i>Gyps bengalensis</i>	A13	08-09-13	Adult, Female	Death due to shock probably
<i>Gyps indicus</i>	D17	07-10-13	Juvenile, Female	Emaciation and muscle atrophy with impaction of proventriculus with a 10x6cm mass of coire fibre (used for perching and cots)
<i>Gyps bengalensis</i>	D50	09-06-14	Adult, Female	Cause unknown
<i>Gyps bengalensis</i>	A35	30-10-14	Adult, Male	Cause unknown
<i>Gyps bengalensis</i>	B80	23-01-17	Adult, male	Septicaemia following bilateral chronic bumble foot - first treated in Sep 2013. Complete local AB resistance. Surgery
<i>Gyps bengalensis</i>	G23	31-01-17	Adult, Unknown	Rescued. Likely very old. Lying on keel - severe articular gout in feet on radiography - did not respond to allopurinol treatment

### Release plans

Dummy satellite telemetry tags were placed on six WRVs at health checks in 2013. These birds were re-caught in 2015 for close examination of the harnesses, tags and birds. One antenna was damaged but there were no lesions to any birds. Birds selected for release will be fitted with similar harnesses. Release plans have been hampered by permissions for satellite tagging, but pre-release aviaries and intensive advocacy work in the surrounding areas has gone ahead. A trial release of two less threatened Himalayan griffon vultures went ahead in 2016, engaging government officials in the process but highlighting the need to tag any Critically Endangered species prior to release.

### Advocacy

Lobbying South Asian governments led to national bans on the sale of diclofenac for veterinary use in 2006 in India, Nepal and Pakistan (and in 2010 in Bangladesh) and further measures being taken in 2015 to reduce opportunities for substituting diclofenac preparations sold for use in people (a ban of multi-dose vials of human formulations in India). Veterinary technical input has played a role, especially in providing expert opinion on the use of NSAIDs in domestic livestock and the threat they may pose to scavenging birds, leading most recently to a national ban on an NSAID other than diclofenac (ketoprofen in Bangladesh).

### Conclusion

Whilst the immediate threat of extinction appears to have been averted, and much progress has been made to make the environment safer for these birds, the long-term future for *Gyps* vultures (and other scavenging birds) in South Asia seems far from assured. There is increasing evidence of the toxicity of other NSAIDs to *Gyps* vultures, other vultures, and other raptors. There remains only one recognised safe alternative NSAID (meloxicam), preparations of which are not always favoured by veterinary professionals in the Indian sub-continent. Safety testing ahead of licensing might help avert further disastrous impact, but as demonstrated more recently in Europe, this is not always adequately considered.

### Acknowledgements

We would like to thank Gidona Goodman, Romain Pizzi, Yedra Feltrer, Devojit Das, Melissa Nollet, and Thalita Calvi for their personal contributions to the programme over the years.

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## PERIODONTAL DISEASE IN THE LESSER BUSHBABY (*GALAGO MOHOLI*)

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Periodontal disease in non-human primates causes important morbidity in managed care. The disease is also a significant issue in human dental practice. Despite gingivitis and periodontitis being two of most common human oral pathologies, they remain poorly described in prosimians. The goal of this review is to illustrate the collaboration of human dental medicine and veterinary medicine in the diagnosis and treatment of two sibling female and one unrelated male lesser bushbabies (*Galago moholi*) with severe chronic gingivitis and periodontitis. Periodontitis was characterised by excessive calculus accumulation, gingival friability and recession, and dental mobility. In the case of one of the females abnormal feeding behaviour was noted, whereas in the other female and male signs were limited to anorexia and weight loss. For all of these animals novel treatments including some adapted from human dentistry were applied. These approaches included topical chlorhexidine varnish and sub-gingival application of minocycline microspheres. Systemic treatments included sub-antimicrobial doses of doxycycline. In addition, directed laser therapy was used on affected gingival tissue for its bactericidal and anti-inflammatory effects. Finally, a proactive, intense regimen of dental prophylaxis and diet and enrichment modification has been instituted with positive results.

## SCREENING PERUVIAN BLACK SPIDER MONKEYS (*ATELES CHAMEK*) AS PART OF A REINTRODUCTION PROGRAMME INTO THE TAMBOPATO NATIONAL RESERVE IN PERU

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In the Tambopata National Reserve, south of the Madre de Dios River in Peru, the black spider monkey (*Ateles chamek*) population has been vanished due to illegal pet trafficking and bush meat hunting. Therefore, a reintroduction programme has been set up and as part of this programme the BPRC has screened groups of *Ateles chamek* for their viral status.

Blood was taken from confiscated monkeys at the Taricaya Rescue Center and shipped on Whatman paper filters to the BPRC. At the BPRC, antibodies and DNA were isolated from the filters and used in ELISA and PCR, respectively.

ELISA results from the spider monkeys were compared with historical data from 36 spider monkey samples and > 1,300 new world monkeys samples from different European zoos.

ELISA screening revealed animals that were positive (or had cross-reacting antibodies) for various Human Herpes-, Influenza A-, Respiratory Syncytial-, Pox-, Dengue-, West-Nile-, Rabies-, Hepatitis A- and Hepatitis B virus.

Most interesting PCR results were reactions with Herpes Simplex Virus 1 (HSV1), Adenovirus and Lymphocryptovirus. Interestingly, HSV1 has also been detected in three species of howler monkeys (*Alouatta seniculus*, *-sara*, *-caraya*), two species of marmosets (*Callithrix jacchus*, *-pygmaea*), white-faced sakis (*Pithecia pithecia*) and golden lion tamarins (*Leontopithecus rosalia*). Poxvirus was found in the common marmoset (*Callithrix jacchus*) and the goeldii monkey (*Callimico goeldii*). As a result of the screening two groups of *Ateles chamek* were released.

During this presentation all relevant data, related to the screening and release of *Ateles chamek*, will be discussed.

## SLEEPING IN TREE HOLES MAY PROMOTE PARASITISM IN WILD LEMURS

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Examining parasite distribution in wild populations improves our understanding of transmission modes of wildlife pathogens, given the effects of species-specific ecological factors. The Milne-Edward's sportive lemur (*Lepilemur edwardsi*) and the Western woolly lemur (*Avahi occidentalis*) are endangered Malagasy primates, which match in activity pattern, feeding ecology, body size and sociality, and cope with the same climatic conditions, but differ in sleeping site ecology. Sportive lemurs sleep in tree holes and individuals show high sleeping-site fidelity, whereas woolly lemurs sleep on open branches and shift their sleeping sites more often. We compared these two sympatric primates, which differ in their sleeping site choice but are otherwise ecologically similar. We collected 86 faecal samples from 26 sportive lemurs and 74 faecal samples from 22 woolly lemurs during both, the dry and the rainy season at the Ankarafantsika National Park in north-western Madagascar. Sportive lemurs were caught directly in their tree holes before being immobilised with 10 mg/kg ketamine (Ketanest®) and 0.5 mg/kg xylazine (Rompun®). Woolly lemurs were remotely immobilised with the same drug combination by using a blowpipe and 1 ml cold air pressure darts (Telinject®). We additionally collected ectoparasites from each individual. Faecal samples were examined by the flotation method using saturated sodium chloride. Using the statistical program "R", GLMMs were constructed to test the influence of the variables "species", "sex" and "season" on the probability of being infected. To account for the fact that many animals were sampled more than once, animal ID was included as a random factor. From the 26 tree hole-sleeping sportive lemurs sampled, 21 % of the individuals excreted eggs of strongyle nematodes and 64 % excreted oxyurid eggs. There was no statistically significant difference in excretion pattern between seasons. Furthermore, 19 % carried ticks (*Haemaphysalis lemuris* and *Ornithodoros* sp.) and 75 % carried mites (*Aetholaelaps trilyssa*), but only in the rainy season. From the 22 openly-sleeping woolly lemurs, 21 % excreted strongyle eggs, also this was limited to the rainy season; no other endo- or ectoparasites were detected. No sex differences in parasite infection were observed in any of the studied lemur species. Findings suggest that species-specific differences in parasite prevalence and diversity may be linked to sleeping site ecology. The higher prevalence and greater parasite species richness in sportive lemurs may be attributed to a favourable environment presented by tree holes for infective stages, facilitating parasitic transmission of both endo- and ectoparasites. Seasonal differences in parasite infection may be linked to environmental factors (here: humidity) shaping survival and development of parasites. They may also be linked to host's reproduction in case of the ectoparasites, since lactation period of sportive lemurs coincides with the beginning of the rainy season. This might be explained by the benefit that parasites get from the appearance of new host after births. This study enhances our sparse knowledge on host-parasite-environment interactions in the tropics and adds valuable information to the limited number of parasitological studies in these endangered primate species.

## YOU ARE WHERE YOU EAT: HIGH *BRUCELLA* SEROPREVALENCE IN COASTAL BUT NOT INLAND ARCTIC FOXES

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The arctic fox (*Vulpes lagopus*) is the only native terrestrial mammal in Iceland. The population comprises both “coastal” and “inland” fox ecotypes, with regard to food resources. While coastal ecotype foxes mainly feed on sea birds and eggs, invertebrates and marine mammal carcasses, the inland foxes feed on ptarmigans, migrating waterfowl, eggs and wood mice. Because of the relatively low biodiversity within arctic ecosystems and the involvement of the species in both marine and terrestrial ecosystems, Icelandic arctic fox population could serve as sentinels for overall ecosystem health of Iceland. It was recently demonstrated that high levels of mercury are present in coastal arctic foxes, but negligible amounts were found in inland populations. This study indicates that the two ecotypes are quite separate and distinct which may indicate that pathogen presence or prevalence between them may also radically differ. We tested serum samples from 39 arctic foxes for the presence of antibodies to *Francisella tularensis*, *Coxiella burnetti*, three *Leptospira* species and *Brucella abortus*. A seroprevalence of 51.2 % to *Brucella abortus* antigens was found, whereas no antibodies were detected against the other pathogens. There was no difference between gender and age groups in the exposure to *Brucella*, however coastal ecotype foxes had a significantly higher seroprevalence than inland ones (62.9 % and 25 %, respectively). Iceland is officially considered to be “brucellosis-free”, marine mammal species in the region, however, were found to be positive for this pathogen. Since coastal ecotype foxes had a significantly higher seroprevalence, it is likely that a spill-over from marine mammals to arctic foxes might take place. We are performing molecular analysis of tissue samples in order to better understand the bacterial species involved and the epidemiology of the brucellosis in Iceland.

**PARANANNIZZIOPSIS AUSTRALASIENSIS IN TUATARA**

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*Paranannizziopsis australasiensis* (*P.a*) has recently been diagnosed in Tuatara (*Sphenodon punctatus*) at two captive facilities in New Zealand. This emerging fungal pathogen is a member of the onygenalean fungal group formally known as *Chrysosporium* anamorph of *Nannizziopsis vriesii*. Infections in tuatara result in superficial brown-yellow exudative to ulcerative dermatitis lesions on the ventrum and gular regions. Fungi of this genera are thought to act as primary pathogens in reptiles; closely related fungal species such as *Ophidiomyces ophiodiicola*, have caused significant morbidity and mortalities in wild reptile populations. The detection of this disease is of concern to wild and captive population health and has resulted in a temporary cessation of tuatara breed and release programmes. The origin of infection and the prevalence of this organism in wild and captive reptile populations in New Zealand is currently unknown.

This research aims to investigate the epidemiology of *P.a* in tuatara. Skin samples were tested for presence of *P.a* by fungal culture followed by PCR and by loop-mediated isothermal amplification (LAMP). Soil samples were analysed by LAMP to determine the presence of *P.a* within the environment. The sensitivity and specificity of these tests has not been determined. Results to date show a -20-40 % prevalence of *P.a* in tuatara when using the LAMP test. Lesions in most cases are superficial, but some positive animals are asymptomatic. *P.a* DNA has been detected in the soil of tuatara burrows, and in enclosures of gecko species. Five island populations, 3 translocated mainland populations and 9 captive populations were included in this study. Further testing is currently being undertaken in other regions to better characterise the distribution of *P.a* in New Zealand.

## **COMPUTERISED TOMOGRAPHY GUIDED ENDOSCOPIC SINUS SURGERY IN AN ADULT WESTERN LOWLAND GORILLA (*GORILLA GORILLA GORILLA*)**

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Chronic rhinosinusitis with nasal polyposis is an important diagnosis in humans and is often an outcome of allergic rhinosinusitis. A 35-yr-old male gorilla with a 4-5 month history of chronic rhinosinusitis presented with prominent facial eyelid edema and bilateral nasal discharge. The nasal discharge was persistent despite oral corticosteroid therapy and directed antibiotics based on bacterial culture and sensitivity. The patient continued to have symptoms, including declining health and increased pain which was addressed by computed tomography (CT) and surgical management. Preoperative planning for an image guided surgery was performed using a limited, non-contrast CT. CT showed all paranasal sinuses as involved with a soft tissue infiltration that supported the diagnosis of infectious rhinosinusitis with nasal polyps and not neoplasia. Bilateral endoscopic sinus surgery was accomplished using stereotactic computer-aided navigation for endoscopic sinus polyp removal with a post-operative diagnosis of chronic rhinosinusitis with nasal polyposis. An inspissated mucopus throughout the bilateral maxillary sinuses extending deep into the bilateral ethmoid and sphenoid sinuses was removed and the soft tissue papilloma-like mass in the nasopharynx resected completely from the left posterior aspect of the inferior turbinate. The image guidance system proved critical for this procedure due to the comparative anatomic landmarks being different from humans. The bilateral endoscopic sinus surgery was replicated approximately one year later when the symptoms returned. The patient remains without symptoms two years after the second surgery. Advanced diagnostic imaging with CT and endoscopic surgical therapy facilitated complete resolution of this bacterial rhinosinusitis with nasal polyposis.

## AN UPDATE ON CARDIAC DISEASE OF GREAT APES – ACHIEVEMENTS AND FUTURE DIRECTIONS OF THE APE HEART PROJECT

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### Summary

Cardiovascular disease is often reported as a significant cause of death among captive great apes. However, our understanding regarding these disorders and consequently our abilities to treat and prevent them are limited. In particular, very little work has been done looking at the European population of apes. The Ape Heart Project (based at Twycross Zoo) is an EAZA Great Ape TAG endorsed initiative striving to develop a collaborative and co-operative approach to the investigation of cardiovascular disease among the European captive great ape population.

As part of this project a systematic review of the zoological and veterinary literature of great apes from 1990 to 2014 was conducted. This was the first review of the great ape literature published since 1990 and the first-ever systematic literature review of great ape morbidity and mortality. Retrospective reviews of mortality among the European zoo-housed western lowland gorilla (*Gorilla gorilla gorilla*), chimpanzee (*Pan troglodytes*), bonobo (*Pan paniscus*) and orang-utan (*Pongo pygmeus* and *Pongo abelii*) populations from 2004-2014 were also carried out. Data from 681 deaths were analysed.

A more detailed review of the various cardiovascular pathologies reported and exploration of possible risk factors is underway. The work of the Ape Heart Project is ongoing, and aims to further understanding about various aspects of great ape cardiovascular disease.

### Introduction

Cardiovascular disease is often reported as a significant cause of death among captive great apes. However, our understanding regarding these disorders and consequently our abilities to treat and prevent them are limited. (LAMMEY *et al.*, 2008; MCMANAMON *et al.*, 2012; MEEHAN and LOWENSTINE, 1994; SCHULMAN *et al.*, 1995, VARKE, 2009) In particular, very little work has been done looking at the European population of apes. The Ape Heart Project (based at Twycross Zoo) is an EAZA Great Ape TAG endorsed initiative striving to develop a collaborative and co-operative approach to the investigation of cardiovascular disease among the European captive great ape population. The Ape Heart Project works in close collaboration with the Great Ape Heart Project (based at Zoo Atlanta, USA).

### Materials and methods

A systematic review of the zoological and veterinary literature of great apes from 1990 to 2014 was conducted. This was the first review of the great ape literature published since 1990 and the first-ever

systematic literature review of great ape morbidity and mortality. A total of 189 articles reporting on the causes of morbidity and mortality among captive great apes were analysed. (STRONG, 2016)

Retrospective observational reviews of mortality among the European zoo-housed western lowland gorilla (*Gorilla gorilla gorilla*), chimpanzee (*Pan troglodytes*), bonobo (*Pan paniscus*) and orang-utan (*Pongo pygmeus* and *Pongo abelii*) populations from 2004-2014 were also carried out. Data from 681 deaths were analysed (STRONG, 2017a; STRONG, 2017b). A more detailed retrospective review of the various cardiovascular pathologies reported and exploration of possible risk factors is currently underway.

Standardised protocols for post-mortem examination and sampling of heart were developed. A total of 34 great ape hearts have undergone detailed macroscopic and histopathologic examination using these protocols so far. The pathologies observed have been characterised and compared to those seen in domestic animal species and humans. The investigation of potential aetiologies (including viral infection, vitamin and mineral deficiencies) is also underway.

Clinical investigations include: electrocardiograms in anaesthetised and conscious great apes, echocardiographic examinations, serum biomarker analyses and a comparative anaesthesia study.

A bank of biological samples (including formalin fixed, frozen and RNAlater preserved heart tissue, blood, serum and urine) and clinical data has been collated to facilitate future collaborative research in this field.

## Results and discussion

Idiopathic and infectious diseases along with disorders of the cardiovascular, respiratory, and gastrointestinal body systems were particularly prominent within the great ape literature during 1990–2014. The literature review concluded that there was a critical need for an up to date review of mortality amongst the European zoo-housed great ape population to be performed.

Mortality reviews were carried out for all great ape taxa, and results were or are going to be published. These identified cardiovascular disease as one of the main causes of death in all great apes, though the frequency of cardiac disease varies between species. Great variation between zoological institutions was observed with respect to the frequency, consistency and quality of pathological examinations and reports, as well as to the vocabulary and terminology used in the pathological descriptions and diagnoses. Standardisation of post-mortem data collection would facilitate future studies.

The work of the Ape Heart Project is ongoing, and aims to further understanding about various aspects of great ape cardiovascular disease. For this it needs the continual support of zoos and sanctuaries across Europe and worldwide. Further details relating to the contribution of samples or data to the project are available on the project's website (<https://twycrosszoo.org/conservation/research-at-twycross-zoo/current-research/ape-heart-project/>).

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## GENE ANALYSIS OF HEPCIDIN AND BLOOD LEVELS IN THREE DIFFERENT LEMUR SPECIES

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The various lemur species have different sensitivities to iron storage disease (ISD). Mutations in the hepcidin gene, the iron regulating hormone, and levels were identified causing ISD in humans. The aim of the current study was to measure hepcidin blood levels and compare the amino acid sequences (AAS) in ringtailed (*Lemur catta*), and red-ruffed lemur (*Varecia rubra*).

Heparin blood was collected during routine health examinations or when health status required further diagnostics. Hepcidin levels were measured by Hepcidin-25 ELISA, (DRG GmbH, Germany). DNA was isolated from blood or liver using Qiagen kit® and the genome was sequenced by high seq Illumina®sequencer. The genomes were mapped to mouse lemur (*M. murinus*) reference genome and partially annotated to identify the hepcidin precursor gene.

The mean values of hepcidin in healthy *L. catta* were significantly higher 18.9 (17.5 - 30.4) than in animals with low packed cell volume (PCV) 11.7 (4.4 – 27.1). Hepcidin levels of healthy *V. rubra* were similar to the ringtailed lemurs with low PCV 12.4 (11 – 15.6) ng/ml. Genetic comparison of the hepcidin gene revealed differences in eight positions between the grey mouse lemur reference gene and the analysed ringtailed and red ruffed lemur sequences. Differences at two positions were found within the active peptide between ringtailed and red ruffed lemurs.

The present study shows that the hepcidin AAS sequence is very conserved and the protein is fully functional in the analysed lemur species. Rather differences in hepcidin levels may cause the increased ISD susceptibility in red ruffed lemurs.

## A FULL MOUTH SPECULUM FOR ELEPHANTS FACILITATING BRONCHOSCOPY, GASTRIC TUBING, AND DENTAL HEALTH EVALUATION

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Here we present the construction of an oral speculum (mouth opener) for the application in Asian (*Elephas maximus*) and African elephants (*Loxodonta africana*) during standing sedation. The steel construction consists of two bite plates connected to threaded poles which can be winched apart. The oral speculum is placed into the mouth gap, resting on the gums in front of the molars. Once the jaws are fully spread, an examination of the oral cavity, the palpation of the glottis and the placement of an endoscope into the trachea or a tube into the oesophagus are possible. Useful applications may be stomach tubing (colic, tetanus), molar corrections, gastric-, tracheal- and bronchial endoscopy. The mouth opener was successfully applied in 19 Asian elephant cases and once in an African elephant. Main reasons for application were dental check/correction ( $n = 2$ ), stomach tubing for tetanus treatment ( $n = 3$ ), bronchoscopy and/or gastroscopy, bronchio-alveolar lavage (BAL), plus stomach fluid collection for *Mycobacterium tuberculosis* culture and PCR ( $n = 15$ ). For the latter approach, once the elephant was sedated (Detomidine/Butorphanol combination), a portable, battery driven 3.3m flexible video endoscope (ESO Endoskopietechnik, Germany) was placed manually and 50-100 ml sterile saline were administered through a catheter into bronchi and aliquots were aspirated immediately. Samples were tested at the respective National Mycobacterium Reference Laboratory, and so far in two incidences, atypical mycobacteria were diagnosed. Especially the access into both, lungs and stomach using the mouth opener, could be of interest in terms of sample collection for mycobacterial culture in elephants.

## EVALUATION OF METABOLIC BONE DISEASE IN WILD AND CAPTIVE COMMON SPADEFOOT TOADS (*PELOBATES FUSCUS*) BY COMPUTED TOMOGRAPHY, AS PART OF A CAPTIVE REARING PROGRAMME

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Through most of its Northwestern range, the European common spadefoot (*Pelobates fuscus*) is in decline. To save this amphibian species from extinction in the Netherlands, RAVON Foundation and ARTIS Amsterdam Royal Zoo initiated an ongoing collaborative captive rearing program in 2012 (STRUIJK *et al.*, 2014). Evaluating whether this species needs UVB-radiation and/or dietary supplementation for healthy bone development is a crucial part of these conservation efforts. The bone mineralization in femurs and skulls of captive and wild common spadefoot toads were measured in Hounsfield units (HU) by computed tomography. Captive group 1 (n = 21) was reared without exposure to UVB radiation, but with dietary supplements. Captive group 2 (n = 18) was reared and kept in an outdoor breeding facility with availability to direct sunlight, but without dietary supplements. Group 3 were wild specimens (n = 12). No significant difference was found between the HU values in the femurs of adults of both captive groups and the wild animals ( $p = 0.512$ ;  $p = 0.165$ ). These findings suggested that this nocturnal and mainly subterrestrial species was not sensitive to develop metabolic bone disease in both captive settings. The significantly higher HU values in the femurs of the group 1 adult animals than in the femurs of the group 2 adults ( $p = 0.009$ ) indicated that dietary supplements did seem to have a positive effect on the bone mineralisation in this species.

### Acknowledgements

The authors thank Lonnet Kunst, Maartje Passon-Vastenburg, Ilse Tiemessen and all other staff at the orthopedic and radiology department of the Medisch Centrum voor Dieren, Amsterdam, and Wilbert Bosman (RAVON), Christian Göcking, Norbert Menke (both NABU-Münsterland), Arno Geiger (LANUV NRW), Michael Bisping and Franz Kraskes (both Münster breeding station). We also thank all keepers and management at ARTIS Amsterdam Royal Zoo, Amsterdam, The Netherlands.

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## ULTRASOUND ASSESSMENT OF WILD OLMS (*PROTEUS ANGUINUS ANGUINUS*) IN CROATIA

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The olm (*Proteus anguinus anguinus*) is a neotenuous salamander species with high life expectancy and low reproductive rate, classified as vulnerable by the IUCN. Little is known about olm health and reproduction in the wild. Using ultrasound biomicroscopy (UBM), we assessed inner organs of wild olms. Data were compared to those obtained previously in 13 captive olms at Zagreb Zoo.

Two divers caught 20 olms in Rupećica, Croatia, during the breeding season in January 2017. We examined internal organs using UBM (30-70 MHz; Vevo 2001, Visualsonics, Canada). Examinations took 10-15 minutes per individual, after which they were re-released to their original habitat.

Body masses ranged from 9-35 g (average  $24 \pm 3$  g). Seventeen olms presented with well-developed gonads. We identified eleven male and six female individuals, whereas the genders of three remain undetermined. Testes and follicle diameters ranged from 1.8 - 3.6 mm and 0.4 - 0.9 mm, respectively. No pathologies were detected. Digestive tracts were well-filled; maximal gall bladder diameters ( $6.0 \pm 2.1$  mm, range 2.3-9.4 mm) were smaller than in the captive population ( $7.1 \pm 1.5$  mm, range 5.0 - 9.2 mm), implying that the two-weekly feeding intervals in captivity may be shortened. Heart rates of wild olms were slower ( $24.2 \pm 2.7$  vs.  $42.9 \pm 4.6$  bpm) compared to captive individuals. Also, gills were shorter, indicating higher oxygen concentrations in the wild.

The assessment of individuals from the wild is important to identify health issues in captive individuals, improve husbandry, and understand normal reproductive development, crucial to establish self-sustaining *ex situ* populations for future captive breeding programmes in this charismatic species.

## EVALUATION OF A PRESSURE-SENSITIVE WALKWAY SYSTEM FOR GAIT CHARACTERISATION IN HUMBOLDT PENGUINS (*SPHENISCUS HUMBOLDTI*)

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Pododermatitis, degenerative arthritis, and other musculoskeletal diseases are common causes of lameness and discomfort in penguins under professional care (DUNCAN *et al.*, 2016; NASCIMENTO *et al.*, 2015). Multiple treatment modalities have been utilised and advocated, but at this time, there is little objective documentation of response to therapy. In domestic mammals, force plates and pressure-sensitive walkways (PSW) have been used to characterize normal and abnormal gait variables (LASCELLES *et al.*, 2006; LASCELLES *et al.*, 2007), allowing for the objective assessment of analgesic treatments (CONZEMIUS AND EVANS, 2012; TEIXEIRA *et al.*, 2016). Limited use in avian species is reported (KURZ *et al.*, 2008; MAKAGON *et al.*, 2015). We evaluated the use of a Tekscan PSW (Walkway 7 System; Tekscan, United States) to obtain quantitative data about normal gait kinematics in Humboldt penguins (*Spheniscus humboldti*). Eight clinically normal adult penguins (mean +/- SD weight: 4.51 +/- 0.34 kg ) were allowed to walk along a ten foot long walkway platform four times. Stance time (AT), stride time (ST), stride length (SL), stride velocity (SV), maximum force (MF), and maximum peak pressure (MPP) were calculated for each foot in each animal. Pairwise comparisons were made between right and left leg in each animal and were not found to be significantly different. Mean values for gait variables were AT: 0.25 s, ST: 0.42 s, SL: 27.14 cm, SV: 67.79 cm/s, MF: 127.14 % BW, and MPP: 107.94 kPa. In addition, we characterised normal static weight distribution across the plantar surface of the feet. Normal values reported here will be used in future studies to assess the use of PSW to detect and quantify lameness and gait alterations in clinically abnormal penguins, followed by controlled objective studies on the efficacy of analgesic therapies.

### Acknowledgements

The authors thank Brookfield Zoo Bird Department and Veterinary Staff for their assistance. We further thank the Grainger Foundation for their financial support of this project.

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## **SUCCESSFUL SEMEN CRYOPRESERVATION IN BLACK-FOOTED PENGUIN (*SPHENISCUS DEMERSUS*) AND GENTOO PENGUIN (*PYGOSCELIS PAPUA*)**

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Among the 18 species of penguins, 11 are threatened with extinction. The gentoo penguin (*Pygoscelis papua*) is currently listed as least concern, but they represent a useful model for optimisation of assisted reproductive techniques in penguin species. The black-footed penguin (*Spheniscus demersus*) is endangered because it is undergoing a very rapid population decline, and thus immediate conservation action is required. Spermatozoa are considered the primary cell type preserved in genetic resource banks to maximise the genetic diversity and ensuing sustainability of captive penguin populations. The purpose of this preliminary study was to adapt and optimize sperm cryopreservation protocols for different penguin species. Semen was collected once weekly, using the massage technique into a microhaematocrit tube in 6 gentoo penguins during April-June, and in 13 black-footed penguins during October-November. Motility parameters were assessed using computer assisted sperm analysis. Propidium iodide and SYBR-14 were used as fluorochromes for the examination of membrane integrity. Semen was diluted using a glutamate-polyvinylpyrrolidone based medium and 8 % glycerol as permeant cryoprotectant, which was added to the diluted samples and equilibrated for 10 min at 5°C. Afterwards, the samples were loaded into 0.25 mL straws and then frozen in nitrogen vapour. Sperm concentration ( $\times 10^6$  sperm/mL) was  $345.5 \pm 216.5$  in gentoo and  $409.7 \pm 272.2$  in black-footed penguin. Freezing-thawing process (fresh vs frozen-thawed) slightly affected the percentage of viable sperm ( $64.8 \pm 6.6$  % vs  $50.5 \pm 6.1$  % and  $88.7 \pm 0.9$  % vs  $44.7 \pm 17.3$  %, in gentoo and black-footed respectively), and the percentage of motile sperm ( $82.9 \pm 3.0$  % vs  $52.1 \pm 6.1$  % and  $66.7 \pm 18.6$  % vs  $36.7 \pm 21.7$  %, in gentoo and black-footed, respectively).

## YIELD OF SEMEN SAMPLES COLLECTED BY URETHRAL CATHETER COMPARED TO ELECTROEJACULATION IN AFRICAN LION (*PANTHERA LEO*)

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Semen collection is not only one of the essential tools for fertility assessment of an animal, but also for the application of artificial insemination with either fresh or cryopreserved semen. There are two different methods described for repeatable semen collection in wild felids, electroejaculation (EE) and, more recently, urethral catheterisation (UC). For this method, a catheter is inserted into the urethra up to the level of the prostate gland. Due to capillary forces, semen that has pooled in the urethra because of muscle relaxation during anaesthesia, will collect into the catheter. In this study, both techniques were applied to nine captive African lions (*Panthera leo*) in a cross-over study to compare standard semen parameters (volume, total motility, progressive motility, sperm concentration and resultant total sperm count).

We found that the ejaculate volume obtained by EE (range: 200-2500µl, median: 950µl) was significantly higher compared to the UC samples (range: 15-600µl, median: 200µl;  $P = 0.0421$ ). Sperm concentration showed the opposite trend, with UC semen concentration (range: 91-6500  $\times 10^6$ /ml, median: 1275  $\times 10^6$ /ml) being significantly higher than the EE sample concentration (range: 63-1300  $\times 10^6$ /ml, median: 262  $\times 10^6$ /ml;  $P = 0.0156$ ). However, the total sperm count, total and progressive motility did not differ between EE and UC samples. These findings are in accordance with a previous comparative study in the domestic cat.

Our results suggest that semen collection via urethral catheter is equally useful for assessing the fertility of lions compared to the established, but more invasive and technically more elaborate method of electroejaculation and provides qualitatively comparable semen samples.

**REPRODUCTIVE PERFORMANCE PARAMETERS IN A POPULATION OF 1,000+ GAME-RANCHED WHITE RHINOCEROSSES (*CERATOTHERIUM SIMUM SIMUM*)**

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The population of free-roaming white rhinoceroses (*Ceratotherium simum*) is under serious threat. Obtaining reproductive reference parameters is a crucial aspect of improving breeding results. In this study performed between 2008 and 2016, reproductive performance was analysed in 1.300 animals kept in a game-ranched environment. Descriptive statistics of this population showed an average annual population growth (%) of  $18.0 \pm 0.1$  (min 5 – max 26). Calving rates were 38 % adult females calved per year. Females had a median age of 83.2 months at first calving (IQR 72.9 – 110.7) and a population inter-calving interval of 29.2 (IQR 24.6 – 34.8) months. Translocations of animals did not interfere with reproductive success in terms of inter-calving periods or age at first calving. Multivariate models showed a clear seasonal calving pattern with a significant increase of the number of calvings during December – April when compared to April – December. Our results did not show any significant skewed progeny sex ratios. Weather observations showed no significant influence of rain or season on sex ratios of the calves. These results have an essential contribution to the population numbers, both wild and captive, and will give a positive input on improving the breeding of this species in zoological institutions.

## BIRTH MANAGEMENT IN WHITE RHINOCEROS (*CERATOTHERIUM SIMUM SIMUM*)

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The white rhinoceros (*Ceratotherium simum simum*) breeding programme has evolved from the poorest to the most successful programmes. Birth rate has increased over the past years to a record high of 22 births in 2016. Alongside the increased number of births, stillbirth and neonate death rate jumped to 22 % over a 5-year period.

Better prediction of birth might be a tool to improve neonate survival in white rhinoceros. In the past, gestational length, reduced food intake and behavioural unrest were vague parameters to predict birth in rhinoceros. In this study weekly blood samples were taken from 11 pregnant females starting 4 weeks prior the estimated date of birth. During the last week prior birth the frequency of blood sampling increased to daily. Mean progesterone concentration in the serum decreased from  $13.1 \pm 1.8$  over  $8.9 \pm 1.0$  to  $5.2 \pm 0.8$  ng/ml 72h, 48h and 24h prior birth, respectively. Decrease of serum progesterone allowed for a timely prediction and close monitoring of events at birth: increased labour activity, break of amniotic fluids, final expulsion of the foetus and times the calf first stood and suckled. As a result ten calves were born live. One calf needed medical intervention to stand up and one was rescued from maternal aggression. Overall, intensive birth management resulted in a reduced neonate death rate in this study of 9 % similar to neonate death rates of 11 % in black and Indian rhinoceros (*Diceros bicornis*, *Rhinoceros unicornis*). It is concluded that serum progesterone monitoring allows timely prediction of the rhinoceros birth. Birth prediction in turn allows tailored birth management and information based decisions on interventions during birth or in the immediate post-partum period.

## THE SABAH RHINO BREEDING PROGRAMME: REPRODUCTIVE PATHOLOGY OF WILD-CAUGHT SUMATRAN RHINOCEROSES AND ITS IMPLICATIONS IN CONSERVATION

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The Sumatran rhinoceros (*Dicerorhinus sumatrensis*) is on the verge of extinction, standing today with less than 100 wild individuals. The Leibniz Institute for Zoo and Wildlife Research (IZW) has been collaborating with the Borneo Rhino Alliance through the use of advanced imaging and assisted reproduction technologies on wild-caught Sabah rhinoceroses (*Dicerorhinus sumatrensis harrissoni*). When captured from the wild, both young cycling females showed severe reproductive pathology that rendered them incapable of carrying a pregnancy, namely extensive cystic endometrial hyperplasia and a large number of uterine leiomyomas. Going back in time, several reports of reproductive pathology in Sumatran rhinoceroses suggest that at least 50 % of wild-caught females were affected by similar conditions. It becomes clear that low densities of a slow-breeding solitary species drastically decrease the possibility of finding a reproductively active partner in the short frame of sexual receptivity, thus leading to long non-reproductive periods in wild individuals. As described for other rhinoceros species in captivity, the organs of non-breeding females are exposed to sex steroids for prolonged periods of time, resulting in a phenomenon known as “asymmetric reproductive aging” which comprises the development of reproductive pathology, depletion of the follicular stock and premature senescence. The distressing discovery of this phenomenon occurring naturally in free-ranging Sumatran rhinoceroses finally explains why wild populations have been continuously declining even when conditions were favourable. Four different techniques for the removal of endometrial cysts were employed with limited success. As natural conception was excluded, the focus of the programme turned to *in vitro* fertilisation.

**CONTRACEPTION IN GIRAFFES USING THE GnRH VACCINE IMPROVAC®**

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Immunisation against GnRH (Gonadotropin releasing hormone) has been extensively studied in horses and boars. We evaluated the effects of the GnRH vaccine Improvac® on testicular and ovarian activity in male (n = 4) and female (n = 14) giraffes (*Giraffa camelopardalis*). The vaccination efficacy was tested through the analysis of faecal hormone metabolite analysis. At the beginning, basic immunisations were applied 3 times in 4 week intervals, followed by booster injections in 4 months intervals (each injection was 3 ml of Improvac®). Faecal steroid analysis revealed falling androgen levels only after the 4th Improvac® injection. Faecal androgens were low for 2 months only and thereafter increased; concentrations fell again after a booster injection. One bull sired a calf shortly after the 3<sup>rd</sup> injection of the basic immunisation, despite falling androgen levels. Comparable results were observed in the female giraffes. A reliable reduction of faecal pregnane levels seem to occur only after 3 – 4 Improvac® injections. One cow delivered a full grown calf despite Improvac® applications starting in the 2<sup>nd</sup> months of gestation. Improvac® in some animals is applied since more than 3 years and reversibility testing is currently underway. We recommend using a 3 times 3 ml regime within 8 weeks for basic immunisation, and to follow with booster injections in 8 – 10 week intervals. Preliminary results were presented at the “35. Arbeitstagung der Zootierärzte im deutschsprachigen Raum, in Rapperswil, Switzerland in 2015. Krawinkel, Schwarzenberger: Der Einsatz von Improvac® bei Giraffen (*Giraffa camelopardalis*) - die ersten Ergebnisse.

## USE OF ASSISTED REPRODUCTIVE TECHNIQUES IN FISH SPECIES FACING EXTINCTION: THE EUROPEAN EEL (*ANGUILLA ANGUILLA*) CASE

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Use of assisted reproductive techniques is widespread and well developed within zoo and wildlife practice with the aim of encouraging captive breeding as a conservation tool. Usually these techniques are focused on facilitating breeding programmes for megavertebrates or highly charismatic species. In the present study we developed a complete reproductive induction programme for the critically endangered European eel.

Different groups of male and female eels were injected weekly with eel-specific recombinant gonadotropins (FSH and LH, Rara Avis Biotec) with the goal of inducing the complete reproductive cycle and obtaining viable high quality gametes.

In males, we compared stimulation with human chorionic gonadotropin (HCG) with specific recombinant FSH, obtaining excellent stimulation results in terms of quantity and quality of sperm production with both protocols.

Females were also divided into two experimental groups, one treated only with FSH and the other with a combination of FSH and LH. Response to hormonal stimulation assessment was done by means of complete physical evaluation (skin color changes, eye enlargement, coelomic distention and weight gain), hormonal profiles and gonad development. Coelomic ultrasound examination, coelioscopy and regular ovarian biopsy were found accurate for follicular monitoring. Information regarding ovarian response and follicular development was essential for adjusting the protocol in terms of hormonal dosage and timing, the latter being vital for correct follicle maturation and ovulation.

Use of biotechnology advancements in association with veterinary techniques, proved extremely useful for advancing the European eel breeding program, serving also as a reference point for other eel species.

## DEVELOPMENTS IN THE EU'S ANIMAL HEALTH LEGISLATION AND EAZA'S NEW POPULATION MANAGEMENT STRUCTURE

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### Developments in EU Animal Health Law

The European Parliament and the Council adopted the Regulation on transmissible animal diseases ("Animal Health Law") in March 2016. It is a framework setting down the principles of European animal health for the next 20 years and replaces more than forty existing regulations and directives, some of which date back as far as 1964, and consolidates provisions on animal health (including aquatic animals) into a single document. The Regulation entered into force on 20th April 2016 and will be applicable throughout the European Union by 2021 (EUROPEAN COMMISSION, 2017). Many impacts are as yet hard to predict, as they will be determined by tertiary (delegated) legislation negotiated during the implementation phase. Regulations on animal welfare will be largely unaffected.

Daniel Nuijten, EAZA's full time lobbyist in Brussels, will discuss the new Animal Health Law, the process by which it will be implemented and its potential impact on zoos and aquariums. He will also touch upon how zoo and wildlife veterinarians can contribute to this process of implementation in their countries.

### EAZA's New Population Management Structure – One size does not fit all!

Danny de Man is EAZA's manager of collection coordination and conservation. His presentation will introduce the new EAZA population management structure that will be implemented from 2018 onwards. Achieving healthy populations of healthy individuals under the one plan approach towards species conservation will only be achieved as part of a team effort and hence the presentation will zoom into the roles and contributions of veterinarians in the EAZA Population Management structure. Pedigree based analyses of the long-term demographic and genetic self-sustainability of EAZA populations, managed as part of an EEP or ESB, have shown that few of these populations can maintain 90 % of genetic diversity for a period of 100 years on their own (LEUS *et al.*, 2011). Similar analysis by other zoo and aquarium associations like AZA and WAZA has reached similar conclusions (BAKER 2007, LEES AND WILCKEN 2009, LONG *et al.*, 2010).

The conclusion that a significant portion of our population management programmes were not yet self-sustainable begged one important question: "so what?" To answer this question EAZA has taken a holistic approach to evaluate the programme structure (EAZA 2017) with the aim to design the EAZA Population Management structure 2.0.

The first step in the process was to develop a vision for animal populations held across the EAZA membership. Where do we want our animal populations to go and what do we want to achieve with them? This resulted in the following vision: EAZA animal populations will contribute to global biodiversity conservation and reconnect people with nature, inspiring them to care for the natural world.

Without animal populations EAZA members cannot contribute to global biodiversity conservation or to reconnecting people with nature. Regardless of whether the conservation contribution takes place *in situ* and/or *ex situ* and is direct or indirect, sound management is required to keep genetically, demographically and behaviourally healthy EAZA animal populations that can achieve the targets that are set for them. Healthy populations of healthy animals is what EAZA aims to achieve with its population management programmes. To be successful it is important that EAZA animal populations are managed scientifically, cooperatively and professionally as well as realistic to their set roles and goals.

Instead of having a fixed 'one size fits all' approach with limited management categories and standard goals and pedigree based analyses it was agreed that EAZA's population management structure must be flexible so as to be able to serve the defined role and meet the defined goal set/priorities for that population.

Intensive management of animal populations is not limited to the EAZA region, or the global zoo and aquarium community. It also happens in sanctuaries, private collections and in protected and non-protected areas in the wild. Therefore, defining roles and goals for population held in EAZA collections is not a process to undertake in isolation. In the future, these efforts will and should be integrated into a joint conservation approach in order to make the biggest impact on species conservation - the so called One Plan Approach (OPA) (IUCN CONSERVATION BREEDING SPECIALIST GROUP 2017).

The IUCN Species Survival Commission Guidelines on the Use of *Ex Situ* Management for Species Conservation (IUCN/SSC 2014) include a five-step decision making process to decide when *ex situ* management is an appropriate conservation tool. The same thinking process can also be used to decide on the role and goals of populations without a (direct) conservation role and will form the basis for regional collection planning in the new EAZA breeding programme structure.

Following from the above, the future EAZA breeding programmes can be defined as population management activities that are endorsed by EAZA for species that are held in EAZA collections aiming towards (maintaining) healthy populations of healthy animals within EAZA or beyond.

Rather than having one general goal of (self) sustainability for EAZA *ex situ* Programmes we move towards a situation where each EEP has its own role(s) and associated population management goals, that will depend on:

- the current status and threats *in situ* and *ex situ* (what do we have and what are current challenges),
- the purpose of that *ex situ* population in terms of direct or indirect conservation or other zoo roles (what do we want),
- the most suitable population management strategy to fulfil the defined role (what do I need to get there) and
- whether that is feasible (feasibility and risk of different scenarios).

The needs of the programme will therefore define programme characteristics.

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## WHEN ELEPHANTS FALL ASLEEP – CASE STUDIES AND PRACTICAL SOLUTIONS FOR GERIATRIC ANIMALS

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Captive management of elephants (*Elephas maximus*, *Loxodonta africana*) has undergone important improvements, and as a result the number of captive aged and geriatric elephants is growing. An important concern is when a geriatric elephant apparently refuses to lie down for resting. Several factors need to be taken into consideration, including degenerative joint and foot diseases (MILLER *et al.*, 2016), social structure, or stressful events (e.g. noise of construction sites) (WILLIAMS *et al.*, 2015; HOLDGATE *et al.*, 2016). Inability or unwillingness to lie down for resting is important from a welfare point of view, as it may impair sleep. We emphasise the importance of lying rest in (aged) elephants by comparing 4 cases from European collections. This is supported by the documentation of positive individual developments in these animals after the instigation of measures that allowed lying rest or an alternative position for standing/leaning rest conducive to sleep. A common denominator of the cases considered is the initial situation where an elephant falls to the ground out of a standing position. Although looking similar to narcolepsy, well known in horses, the disorder has not yet been reported in elephants. Potential preventative measures are proposed, such as sand mounds or logs mounted at walls that allow resting the pelvis. Such measures could help fulfil the requirement for sleep in (aged) zoo elephants and prevent critical situations with recumbent elephants. Moreover, the facilitation of resting time in a satisfying quality might lead to enhanced welfare in zoo elephants (HOLDGATE *et al.*, 2016).

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## THE WELFARE ASSESSMENT QUESTIONNAIRE AT ZURICH ZOO - A TOOL TO MONITOR QUALITY OF LIFE IN GERIATRIC OR CHRONICALLY ILL ANIMALS

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Choosing the moment when euthanasia in zoo animals is warranted can be challenging, especially in geriatric or chronically ill animals, in which processes of deterioration are slow. Based on the paper by LAMBETH *et al.* (2013) a welfare assessment procedure has been developed and implemented since 2016 at Zoo Zurich, to monitor the quality of life in affected animals. The assessment includes a questionnaire and on-site visitations, which are carried out at set intervals. Decision on which animal to include in the assessment is initiated by the curator, headkeeper or veterinarian. The questionnaire is designed for the specific animal and includes between 15 and 25 questions, which are grouped in the following sections: feeding, body condition, behaviour and subjective quality of life assessment. Answers are given as “yes / no” or by grades. The questionnaire is filled out by the headkeeper and sent to the curator and veterinarian. Assessment intervals vary from monthly to every three months for the questionnaire and every three to six months for joint on-site evaluations. We recommend this approach to welfare monitoring as we believe that it serves as a standardised method of assessing the quality of life and the decision making process regarding termination of life based on the following criteria: 1. animal welfare, 2. systematic approach, 3. transparency and 4. objectivity.

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## WHERE DOES THE MONEY GO? A DECENNIAL RETROSPECTIVE ANALYSIS OF DRUG EXPENDITURE AT ZURICH ZOO

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### Summary

Veterinary medicines may significantly contribute to a zoo's veterinary budget and preferences in the usage of various drugs change over time. In an attempt to identify trends in the use of veterinary medicines, a retrospective analysis of drug expenditure from 2006 to 2015 was performed. Pharmacological agents were classified into 13 categories for which a yearly and total expenditure was calculated. These data were analysed for normality with the Anderson-Darling Normality Test and for trends over time with the Mann-Kendall Test. Over ten years, a total of 160'130 Swiss francs (149'435 Euros) were used in the acquisition of veterinary medicines, consisting of approximately 30 % of the veterinary station's budget for the decade. Annual expenditure for veterinary medicines was not normally distributed. Therefore, veterinary managers may have to calculate for significant yearly fluctuations. Overall, anaesthetics and antibiotics accounted for the majority of drug expenditure (14.5 and 13.8 %, respectively). During the decade, no significant change was identified in overall yearly drug expenditure; however, there was a significant increase in the use of contraceptives ( $P < 0.001$ ), probably reflecting the increased practice of contraception in zoo animals. A positive trend was noted in the use of vitamins and minerals ( $P = 0.053$ ) interpreted as an increased awareness of their prophylactic relevance. Finally, a significant increase was noted in the ratio of reserve (i.e. fluoroquinolones, third generation cephalosporins) to first-line antibiotic expenditure ( $P = 0.024$ ). This may be interpreted as a necessity for increased use of reserve antibiotics or simply as an increase in the costs of reserve in comparison to first-line antibiotics. While data are limited to one zoological institution, antimicrobial practices in zoos may deviate from standard recommendations regarding their use (EUROPEAN COMMISSION 2015, UNGEMACH *et al.*, 2006).

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## **A SUSPECTED CASE OF PSYCHOSOCIAL SHORT STATURE IN A FEMALE GORILLA (*GORILLA GORILLA GORILLA*): WHEN APE PSYCHOLOGICAL DISORDERS MEET HUMAN'S**

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ZooParc de Beauval houses a large familial group of 13 individuals of western lowland gorillas (*Gorilla gorilla gorilla*). On the 19<sup>th</sup> of August 2010, a female was born from an inexperienced mother (3<sup>rd</sup> pregnancy with 2 previous early neonatal deaths). Child-mother relationship was monitored closely and strong lack of maternal attention was noticed. The young female had a slow growth rate compared to other juveniles of the same age within the group. On April 2013, changes in her behaviour (lethargy, interaction decrease) and a diarrhea unresponsive to treatments led to a detailed clinical exam. Delayed growth was confirmed (between half and a third of the normal weight) with proportionate body parts. The female was considered suffering from proportionate dwarfism. Differential diagnoses included primary hyperparathyroidism, congenital disorder or psychosocial origin. Biochemistry, hormonal panel, x-rays, ultrasonography and CT scan exams, genetic analysis (karyotype) were unremarkable.

The psychosocial origin was considered to be the primary cause of the dwarfism. In human medicine, psychological short stature (PSS) (also known as Kaspar Hauser Syndrome) is a growth disorder that is observed between the ages of 2 and 15 years old, caused by extreme emotional deprivation or stress (feral children or children kept in abusive, confined conditions). The symptoms are probably due to a decreased production of growth hormone releasing hormone and/or an increased production of growth hormone inhibiting hormone.

The female and her mother were provided supportive care (keepers' attention, more feeding times, toys, etc.) and growth delay decreased while her mother health condition improved and so maternal attention.

This is the first description of a suspected case of psychosocial short stature in a great ape.

### **Acknowledgments**

The authors would like to thank Dr Bénédicte HAETTICH, CHU du Mans, France and the veterinary clinic Advetia, Paris, France.

## VIRULENCE TRAITS, ANTIBIOTIC RESISTANCE AND CLONALITY OF ENTEROCOCCI ISOLATED FROM LIONS (*PANTHERA LEO*) OF THE NIASSA NATIONAL RESERVE, MOZAMBIQUE

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Lions (*Panthera leo*) are classified as vulnerable, according to The IUCN Red List of Threatened Species. In Mozambique, there are well-characterised populations in only two reserves, Niassa National Reserve and Gorongosa National Park. In addition to poaching, decrease in prey availability, and habitat destruction, populations' recovery may be compromised by infectious diseases.

*Enterococcus* are commensal intestinal bacteria, and although animals are not usually affected by enterococcal infections, they can cause severe diseases. Enterococci presence in wild animals, including in African Lion populations, poses apprehension regarding their potential pathogenicity and transmission to other animals and humans, especially considering their intrinsic and acquired antimicrobial resistance ability.

During a health monitoring programme conducted in Niassa National Reserve (Mozambique), 44 lion faecal samples were collected (October-November 2014). Enterococci were isolated, identified and characterised by PCR-fingerprinting. Isolate susceptibility to 19 antibiotics including tetracyclines, beta-lactams, aminoglycosides, cephalosporins, macrolides, cyclic peptides, rifamycins, macrolide-lincosamide-streptogramins, quinolones, fluoroquinolones and sulphonamides, was evaluated.

From 44 lion faecal samples, it was possible to obtain 37 isolates. PCR-fingerprinting allowed their allocation into six genomic groups. Resistance levels ranged from 0 % (amoxicillin/clavulanic acid, chloramphenicol, penicillin, imipenem, high-level gentamycin and streptomycin) to 100 % (nalidixic acid, low-level streptomycin). Resistance to vancomycin was observed in 19 % of isolates. Results were unexpected since resistance development is usually related to antibiotics use, which had not occurred in Niassa lion populations.

Bacterial isolates presented high genomic diversity, assuring its representativeness regarding the diversity of enterococci eliminated to the environment via lion faeces, and confirming their potential as indicators for monitoring resistance dissemination.

## POST-MORTEM EXAMINATION OF FREE-RANGING EUROPEAN BROWN HARES (*LEPUS EUROPAEUS*) FROM SCHLESWIG-HOLSTEIN, GERMANY

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For several years, hunters in Schleswig-Holstein, Germany, have observed an increase of diseased European brown hares (*Lepus europaeus*) associated with an elevated number of deceased animals. During the hunting season in autumn 2016, 60 post-mortem examinations on hunted free-ranging hares (29 females, 31 males) were performed. From each of three hunting grounds, 20 animals were chosen and blood samples were taken immediately after death. During the necropsies within the next 48 hours, selected samples for histopathology were collected. Furthermore, routine bacteriological investigation of small intestines and lungs, as well as a parasitological screening of faecal samples were performed. Liver tissues were negative for tularaemia (tul4-PCR, culture), European Brown Hare Syndrome Virus and Rabbit Haemorrhagic Disease Virus Type 2 (PCR, antigen-ELISA). No antibodies for *Francisella tularensis* were found. Serological testing for EBHSV and RHDV-2 specific antibodies will be performed. Besides hunting-related injuries and agonal alterations, histopathological results included varying findings of eosinophilic, lymphocytic or pyogranulomatous hepatitis, bile duct proliferation, lympho-plasmacellular enteritis, granulomatous-necrotizing steatitis, as well as follicular hyperplasia of the spleen and mesenteric lymph nodes. The parasitological analyses demonstrated marked intestinal coccidiosis in most cases (88.3 %), in addition to gastrointestinal strongylids (41.6 %) and *Trichuris* spp. (18.3 %) occurring in some individuals. *Escherichia coli* were cultured from 48 small intestine and 49 lung swabs. This project contributes to the advancement in preventive protection of public health and food safety. Further investigations should include the examination of deceased animals in order to reveal possible causes of the hunting bag decline of European brown hares.

**POST-MORTEM FINDINGS OF 12 SPERM WHALES (*PHYSETER MACROCEPHALUS*) STRANDED ON THE COAST OF SCHLESWIG-HOLSTEIN, GERMANY, IN EARLY 2016**

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Throughout the last decades, multiple strandings of sperm whales (*Physeter macrocephalus*) have been recorded in countries bordering the North Sea. Between January and February 2016, an especially large stranding event occurred in the North Sea, when a total of 30 sperm whales became stranded in five countries. The majority of them, 13 animals, were found in Germany on Schleswig-Holstein shores and necropsied by the Institute for Terrestrial and Aquatic Wildlife Research (ITAW), Buesum, Germany accordingly. Measurements and photos were taken of all animals, but due to advanced decomposition only 12 sperm whales were necropsied. All organs were investigated and content of the gastro-intestinal tracts was quantified and identified. No evidence of severe trauma or diseases was found.

All sperm whales were immature males, measuring between 10m and 12m in length. Age estimation based on growth layer group counts of the teeth ranged between 10 and 15 years. Blubber thickness and muscle condition indicated a good nutritional status. Metazoan parasites were located on the skin, in the blubber, stomach and intestine, mostly only associated with mild inflammatory lesions. Four animals displayed marine debris including fishing gear, plastic foil and hard plastic fragments in the digestive tract. Additionally, ~110 000 cephalopod beaks and some fish remains were collected, indicating feeding in the North Atlantic prior to stranding. Circulatory failure due to stranding is regarded as the most probable cause of death. Yet, it remains unclear why the animals migrated to the North Sea in the first place.

## HEXABOTHRID MONOGENEAN PARASITES IN A GROUP OF SHORT-TAIL STINGRAYS (*DASYATIS BREVICAUDATA*) AND SUCCESSFUL USE OF A BLOOD TRANSFUSION TO TREAT ANAEMIA

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Two individuals in a group of four Short-tail stingrays (*Dasyatis brevicaudata*) presented with reduced feed intake, pallor and dyspnoea. Large numbers of sanguinivorous Hexabothrid monogeneans were recovered after treatment with oral praziquantel. A ray with a packed cell volume (PCV) of 3 % died despite supportive care. Clinical deterioration and a drop in PCV from 4.2 to 3 % was observed in a second ray. A blood transfusion was performed using a healthy conspecific from the same group as a donor. Both rays were anaesthetised with 2-phenoxy ethanol and a basic cross match performed. Thirty-five millilitres of blood was drawn from the tail vein of the donor into syringes preloaded with heparin and gently rocked. Whole blood was given slowly into the tail vein of the recipient with a 21g butterfly catheter. Marked clinical improvement was seen in the recipient ray day one post transfusion and it began feeding on day two. PCV was 10.6 %, 19.3 % and 20 % at 3, 5 and 8 months' post transfusion. Hexabothrid monogeneans were subsequently identified in the gills of a blue stingray (*Dasyatis chrysonota*) exhibiting no clinical signs in an adjacent tank. Amplification of a 28S gene fragment of monogeneans from *D chrysonota* and *D brevicaudata* was performed and the sequences aligned to existing hexabothrid sequences on Genbank® and previously collected specimens. Initial data suggests that the hexabothrids are two different species in the same or similar genus. Understanding of host parasite relationships has implications for biosecurity and management of mixed species exhibits in aquaria. Blood transfusion may be an effective treatment for severe anaemia caused by heavy parasite load in elasmobranchs.

## **SURGICAL CHALLENGES: A FIFTEEN YEARS' EXPERIENCE**

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The decision process in surgery is usually based on the experience, in terms of repetition of a procedure already applied in similar situations (usually observed in specific recurrent and specialised surgery) and/or adaptation (requiring a general overview of surgery combined with individual characteristics of the species). Frequently, in zoo animal surgery, the surgeon copes with lack of bibliographic references and a low number of similar cases. These conditions influence the preparation process and highlight the relevant presence of a surgeon with a general perspective, aware of the species specificities and possible applicable techniques. Thus, a general zoo surgeon with accumulated experience is an essential piece of a multidisciplinary team even in very specific procedures.

The maintenance of zoo species under human care must involve the adequate availability of resources in terms of equipment, facilities and best-practice surgical techniques. The fact that some of the patients are invaluable in terms of conservation implies the endeavour of establishing these surgical resources. Moreover, these resources tend to be used without exception independently of each patient genetic or conservation value. Given that, once in zoos, the preservation of each animal life should be mandatory and should be one of the benefits of the zoo animals under human care.

The use of surgery as a therapeutic tool is closely related to the early diagnosis and prompt intervention. The fact that generally these species instinctively conceal overt signs of illness until the disease process is advanced should not be neglected. The continuous development of complementary diagnostic tools and its frequent use in zoos have increasingly capacitated surgical intervention as an actual therapeutic option.

Minimal invasive surgery is becoming increasingly more applicable in this field. Among several advantages when compared with conventional surgery, the reduction of the probability of suture dehiscence, the prompt re-integration in the groups of origin without alteration of the social hierarchy and the reduced monitoring in the post-op period represent valuable reasons to its application. Nevertheless, this approach is obviously not indicated for all procedures and requires training and frequent practice in order to be considered in the decision process.

Recent developments in terms of surgical techniques in human surgery can undoubtedly provide some useful resources to zoo animal surgery, but some priorities and aims are also significantly different. Advances in the former field should not automatically represent *per se* a benefit for zoo species. The clear understanding of which should be the key objectives of each procedure is, in the author's opinion, the adequate approach to zoo animal surgery.

## **SURGICAL MANAGEMENT AND IDENTIFICATION OF 3 DIFFERENT NEW TUMORS IN CAPTIVE EASTERN QUOLLS (*DASYURUS VIVERRINUS*)**

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Neoplasms are quite frequent in captive dasyurids as they become older. Three Eastern quolls (*Dasyurus viverrinus*), held at the Ménagerie du Jardin des Plantes (Paris, France), presented a growing mass but with different location.. The first animal was a three-year-old male presenting a mass on the right upper side of the skull associated with a mild exophtalmia on the controlateral side. The mass was non-adherent and entirely removed. Anaesthesia was induced with a mixture of medetomidine (0.06 mg/kg), butorphanol (0.1 mg/kg) and ketamine (5 mg/kg) and maintained with isoflurane by intubation. The surgery and post-operative phase went uneventful (tramadol 5 mg/kg PO BID four days, meloxicam 0.2 mg/kg PO SID five days, amoxicillin 12.5 mg/kg PO BID seven days). Histopathological examinations of the tumor revealed an osteoma. The second animal was a four-year-old female suffering from posterior ataxia associated with a mass on the left side of the marsupium. After a premedication with 0.4 mg/kg of butorphanol, the anaesthesia was induced with isoflurane (mask). After surgical removal, a histiocytic sarcoma was diagnosed. The animal died four days later despite of the post-operative treatment with butorphanol (0.4 mg/kg IM, BID), amoxicillin/clavulanic acid (12.5 mg/kg, IM, BID), meloxicam (0.2 mg/kg, SC, SID). During necropsy, several metastatic lesions were observed on the spleen and on both lungs. The last animal was a two-year-old female presenting chewing problem. A highly infiltrative mass was observed on the left mandible during close clinical examination. Sampling was performed and histological examination revealed a peripheral ossifying fibroma (ossifying fibroid epulis). Surgical treatment was judged impossible and chemotherapeutic treatment was not considered due to the risks of repeated anaesthetics and unknown drug efficacies. The tumor was medically managed with mistletoe extracts (*Viscum album*) in three successive treatments consisting of four strictly sub-cutaneous injections every two days with 0.1 mg, 1 mg and 10 mg respectively. Today, the mass shows a reasonable size to allow a normal feeding. Treatment with mistletoe extracts is regularly used in human and veterinary oncology with interesting results. To the authors' knowledge, this is the first report of these neoplasms in dasyurids and more widely in marsupials.

**EFFECT OF MIDAZOLAM HYDROCHLORIDE ADMINISTRATION VIA INTRANASAL ROUTE ON SURVIVAL IN SURF SCOTERS (*MELANITTA PERSPICILLATA*) CAPTURED FOR IMPLANTATION OF INTRACOELOMIC TRANSMITTER**

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Intracoelomic implantations of satellite transmitters have been associated with suboptimal survival rates in surf scoters (*Melanitta perspicillata*). It has been proposed that physical exertion and stress associated with capture, handling, and confinement of these birds results in physiological alterations that could alter post-surgical survival. The objective of this study was to evaluate if the intranasal administration of midazolam could improve the survival rate of surf scoters implanted with intracoelomic transmitters. Midazolam hydrochloride (Versed 5 mg/ml, Hoffmann-LaRoche-Limited, Canada) - 4.6 – 6.0 mg/kg) was administered intranasally to 26 randomly selected female adult surf scoters shortly after their capture in Forestville (October 2013, Quebec, Canada). The same volume of saline was also given to 26 female adult surf scoters for comparison. All birds were surgically implanted with an intracoelomic transmitter equipped with a percutaneous antenna by the same surgeon. To assess the effect of the treatment, transmitters were programmed to transmit 2h each day for 30 days post-implantation and survival rate was estimated for each group using the telemetry data. The association between the administration of midazolam and survival was assessed while controlling for other factors such as body mass, haematocrit, plasma total solids, duration of surgery, anaesthesia and confinement (which ranged from 190 to 530 min). Death odds at 30 days for the midazolam group (23 %) was significantly lower than those for the saline group (61 %) ( $p = 0.004$ ). No other variable was significantly associated with survival. This result indicates that sedation with midazolam following the capture might increase post-surgical survival in surf scoters.

## PROPOFOL-SEVOFLUORANE ANAESTHESIA IN JUVENILE *VARANUS KOMODOENSIS*

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Neck trauma due to manual restraint during induction via facemask are reported in juvenile *Varanus komodoensis*. Here we report successful induction by injectable anaesthesia in four 1 year-old komodo dragons for ultrasound and laparoscopic examination

For three animals, induction was performed by IV injection of propofol at 5 mg/kg (Lipuro ®, Braun, Rubí, Spain) in the tail under manual restraint. The fourth komodo dragon was premedicated with IM midazolam (0.2 mg/kg, Midazolam B. Braun, Braun, Melsungen, Germany) and medetomidine (0.07 mg/kg Sededorm ®, Laboratorios Karizoo, Caldes de Montbui, Spain), and induced 20 minutes later with propofol (2 mg/kg IV). Anaesthesia was maintained by sevoflurane (3 %) (Sevorane ®, Abbvie S.L.U., Spain) via endotracheal tube, with manual insufflation at 10 breaths per minute on all four animals. Analgesia was provided by intramuscular meloxicam (0.2 mg/kg, Metacam ®, Boehringer Ingelheim Vetmedica GmbH, Germany).

The time to unresponsiveness after propofol injection was similar in all animals (3 - 6 min). Total procedure since induction ranged from 45 to 105 minutes. Spontaneous respiration resumed 7 to 50 minutes after sevoflurane administration ended. Heart rate and SpO<sub>2</sub> were similar for the first three komodo dragons (38 - 44 bpm and 98 - 99 % respectively). Restraint and venepuncture for induction was subjectively easier and shorter in the premedicated Komodo dragon, which also presented lower SpO<sub>2</sub> (90 %) and HR (16 bpm) readings, in agreement with alpha-2 agonist effects described in mammals. Induction with IV propofol provided a reliable and safe anaesthesia for laparoscopy in Komodo dragons, while avoiding the risk of neck trauma during induction with sevoflurane masking.

## SEVOFLURANE ANAESTHESIA IN LIVINGSTONE'S FRUIT BATS (*PTEROPUS LIVINGSTONII*)

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Livingstone's fruit bat (*Pteropus livingstonii*) is an IUCN-listed critically endangered species found on only two islands in the Indian Ocean west of Madagascar. A total of thirty-nine anaesthetic procedures were carried out in captive Livingstone's fruit bats. Twenty-nine anaesthetic procedures were done in twenty-seven, considered healthy, specimens, eight in six specimens suffering from cardiac disease and two in pregnant females. Data from specimens affected from cardiac disease and pregnant females was excluded from the results.

Specimens were anaesthetised for veterinary health checks, including haematology, biochemistry, radiography and echocardiography, using sevoflurane (SevoFlo, Abbot Laboratories, United Kingdom).

Animals were starved for 14-18 hours prior to the anaesthesia. Induction was carried out under physical restraint without any pre-medication, delivering sevoflurane at 8 % by mask. Specimens were intubated and maintained at 4 % sevoflurane and 1.5 litres/minute of oxygen.

The total anaesthesia duration for the healthy specimens was  $44.8 \pm 7.3$  minutes. During anaesthesia, the animals were maintained in dorsal or lateral recumbency for  $36.5 \pm 6.9$  minutes and in a roosting position for  $8.1 \pm 2.6$  minutes. The induction time was  $1.9 \pm 0.5$  minutes, while the recovery took  $7.8 \pm 2.1$  minutes. No anaesthetic complications were observed in any of the specimens.

Significant differences in the core temperature were found between the oesophageal and rectal measurements. The oesophageal temperatures were significantly lower ( $p < 0.05$ ) than the simultaneous rectal measurements and the 95 % limits of agreement were  $+1.65^\circ$  to  $-1.08^\circ\text{C}$ .

Blood samples to check blood glucose were obtained following induction and prior to recovery from anaesthesia. A significant decrease in blood glucose was noted through the anaesthesia. Mean blood glucose was significantly lower at the end of the procedure when compared with the initial sample ( $2.5 \pm 1.2$  mmol/l vs.  $7.5 \pm 3.3$  mmol/l) ( $p < 0.05$ ). The time interval between samples was  $40.2 \pm 7.7$  minutes, but differences were not related to the duration of anaesthesia ( $p > 0.05$ ,  $R^2 = 0.0835$ ).

Although additional research is required in some physiological aspects related to anaesthesia, sevoflurane appears to be a safe and reliable anaesthetic agent for Livingstone's fruit bats in a variety of clinical presentations.

## EFFECTIVE THIAFENTANIL IMMOBILISATION AND PHYSIOLOGICAL RESPONSES OF FREE RANGING MOOSE (*ALCES ALCES*) IN NORTHERN SWEDEN

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Eleven (six males and five females) free-ranging adult moose (*Alces alces*) were darted from helicopter with thiafentanil (7.5 mg per animal) during March 2014 in Northern Sweden. Physiological evaluation was performed based on vital signs and blood gases. Arterial blood was collected after induction and again after 10-15 minutes of intranasal oxygen therapy (3L/min) and analyzed immediately with an i-STAT<sup>®</sup> 1 portable analyzer (Abbott Laboratories, Illinois, USA). For reversal, all animals received 10 mg naltrexone per mg of thiafentanil, half intramuscularly and half intravenously. All were sufficiently immobilised with a single dart injection. Induction occurred in  $2.7 \pm 1.0$  minutes after successful darting. One individual became recumbent while crossing a river and was immediately given reversal drugs. Animals maintained sternal recumbency with the head raised. Vital signs were stable throughout immobilisation. Nine of ten moose were hypoxemic before oxygen administration, with seven animals being markedly hypoxemic ( $\text{PaO}_2 = 40\text{-}59$  mmHg). The mean  $\text{PaO}_2$  increased significantly after oxygen therapy ( $p = 0.01$ ), but six moose still had  $\text{PaO}_2$  values below 80 mmHg post-treatment. All moose were acidemic ( $\text{pH} < 7.35$ ), with nine showing marked acidemia ( $\text{pH} < 7.20$ ). The mean pH increased significantly between samples ( $p = 0.01$ ). Hypercapnia was reported in all ten moose, with eight having marked hypercapnia ( $\text{PaCO}_2 > 60$  mmHg). Lactate levels were high in all animals, but decreased significantly with time ( $p < 0.01$ ). Recoveries were calm and without noticeable side effects. Mean time from reversal to standing was  $1.8 \pm 0.7$  minutes. Monitoring of animals' physiological responses to immobilisation as well as oxygen supplementation for correction of hypoxemia are recommended in this species when using this regimen.

## LONG-TERM MANAGEMENT OF TYPE 2 DIABETES MELLITUS IN CALLITRICHIDS WITH ORAL ANTI-HYPERGLYCAEMIC MEDICATION AND DIETARY MODIFICATION

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Type 2 diabetes mellitus (T2DM) is the most common form of spontaneous diabetes in humans and nonhuman primates and is frequently reported in callitrichids. A study of DM in primate populations in zoological institutions found that 27.6 % of diabetic primates were New World primates, of which 68.8 % were callitrichids, and reported that 19 % of zoos did not treat diabetic animals (KUHAR *et al.*, 2013). Oral anti-hyperglycaemic drugs are the first line of therapy for T2DM in humans along with dietary changes. There are no publications on the successful use of oral anti-hyperglycaemic medication in callitrichids. We report on the diagnosis of five cases of T2DM in callitrichids based on persistent hyperglycaemia, glucosuria and elevated serum fructosamine (STRIKE and FELTRER, 2017). Diets were modified to reduce simple carbohydrates, eliminate high-sugar content fruit and to increase protein, fibre and fat for a steadier source of energy but were not successful in managing the condition alone. Treatment with metformin monotherapy (100 - 200 mg/kg orally sid - bid) was successful in controlling and maintaining normoglycaemia in three individuals for between 4 and 6 years (Metformin, Relonchem Ltd, Cheshire House, Gorsey Way, Widnes, Cheshire, WA8 0RP, UK). The addition of glipizide (1 mg/kg orally bid) was required to achieve glycaemic control in a fourth animal (Glipizide, Mylan, Potters Bar, Hertfordshire, EN16 1TL UK). A fifth animal refractory to treatment was diagnosed with a poorly differentiated adenocarcinoma of unknown cellular origin that had invaded the pancreas and other abdominal organs. Compliance to medication was good in all animals and no side effects were seen. Based on these findings, we suggest that anti-hyperglycaemic drugs, together with appropriate dietary changes, can be effective in the long-term treatment of T2DM in callitrichids.

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## STABLE ISOTOPE SIGNATURES OF PUFFINS (*FRATERCULA ARCTICA*) AND COMMON MURRES (*URIA AALGE*) ON KNOWN DIETS – A VERIFICATION STUDY

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The Puffin (*Fratercula arctica*) is in decline due to massive breeding failures in the North Atlantic. The ecological mechanisms, which likely include alteration of pelagic food webs associated with climate change and fisheries, can be elucidated by studying wild bird diets. Stable isotope values of carbon and nitrogen in predators reflect those of their prey, with the adjustment of a discrimination factor, providing estimates of diet composition of an individual. However, discrimination factors are species- and diet-specific, and few data exist, particularly for seabirds. Controlled feeding experiments in which the isotopic value of the food is known are needed to produce accurate estimates of the discrimination factor. Zoos are uniquely positioned to provide such data, demonstrating yet another way that zoos may contribute to species conservation.

Following approval from the zoo's Animal Care and Use committee, zoo-housed puffins (n = 7) and common murrelets (*Uria aalge*, n = 7) were sequentially subjected to four four-week periods of selective feeding of a single food source. The diet items offered were capelin, sandeel, krill and herring. After each four-week period, birds were manually restrained for collection of two primary wing feathers and a blood sample. Subsequently, blood and feather samples, and representative samples of each diet item were dried, finely ground and analysed for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . Each of the four diets produced isotopically distinct results using blood, while feathers represented a mixed diet composition, likely representing the nutritional regime during the last moult of the birds. This study provides the necessary "grounding" to establish SIA as an effective tool in monitoring the diets of wild auks.

## HELP! MY BEAR IS GOING BALD! RECENT ADVANCES IN THE TREATMENT OF ANDEAN BEAR (*TREMARCTOS ORNATUS*) ALOPECIA SYNDROME

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Andean bear (*Tremarctos ornatus*) alopecia syndrome (ABAS) commonly affects captive bears, especially sexually mature females. Typically, ABAS presents with progressive bilaterally symmetrical flank alopecia with variable pruritus and secondary bacterial and *Malassezia* infections. Recent work suggests it is an immune mediated condition. Hitherto there has been no effective treatment. Here, we describe successful long-term management of four cases of ABAS. Skin biopsies and cytology from the bears showed a mixed dermal inflammatory infiltrate, alopecia, hyperkeratosis, and *Malassezia* dermatitis. Haematology, serum biochemistry, thyroid and adrenal function were normal. One case had positive IgE serology for environmental allergens. There was no sustained response to antifungals, antihistamines, allergen specific immunotherapy and topical antimicrobials. There was a partial response to ciclosporin (Atopica® cat, Novartis Animal Health, U.K.; 5 mg/kg p.o., s.i.d.) in one bear and to oral glucocorticoids (dexamethasone sodium phosphate, [Colvasone 0.2 %, Norbrook, U.K.], 0.15 mg/kg p.o., s.i.d. or prednisolone [Deltacortene, Bruno Farmaceutici, Italy and Megasolone 20, CoopHAVet, France], 0.3 - 1.2 mg/kg p.o., s.i.d.) in three cases but treatment was withdrawn following adverse effects. Treatment of all four bears with oclacitinib maleate (Apoquel®, Zoetis, U.S.A.; 0.46 - 0.5 mg/kg p.o., b.i.d for 14 days and then s.i.d) resulted in rapid complete resolution of pruritus with subsequent fur regrowth and improved demeanour. The oclacitinib was then tapered to the lowest dose that prevented pruritus (0.1 - 0.4 mg/kg p.o., s.i.d.). No adverse effects have been noted. To our knowledge, oclacitinib is the first treatment to result in sustained clinical improvement in ABAS.

## INFLAMMATORY BOWEL DISEASE IN A BENGAL TIGER (*PANTHERA TIGRIS*)

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Inflammatory bowel disease describes a clinical syndrome characterised by idiopathic infiltration of inflammatory cells in the mucosa and submucosa of the gastrointestinal tract. It is classified according to the prevailing type of cells, such as lymphocytic-plasmatic, eosinophylic, neutrophylic, granulomatous or histiocytic. It is an infiltrative gastrointestinal disease commonly diagnosed in dogs and cats that is less frequently found in cattle and horses. However, cases are rarely diagnosed and reported in wild felids.

This work describes a clinical case of a 10-year-old female Bengal tiger (*Panthera tigris*) at Vigozoo, which presented clinical signs of vomiting, diarrhoea and weight loss in November 2011. In the following 15 months, a 40 % weight loss (from 120 to 75 kg) was observed. Laboratory findings showed no significant changes. After ruling out renal insufficiency, intra-abdominal tumours, hyperthyroidism, pancreatitis or an infectious disease, abdominal ultrasounds revealed thickening of the small intestines. A complete intestinal wall biopsy was fixed in formalin and embedded in paraffin for histopathological examination and a diagnosis of inflammatory bowel disease was established. Microscopically, a mixed inflammatory infiltrate was observed in the lamina propria. It was composed mainly of polymorphonuclear eosinophils, plasma cells and lymphocytes. The lymphatic vessels were slightly dilated. The treatment consisted of metilprednisolone (Urbason® 40 mg, Sanofi-Aventis S.A, Barcelona, Spain) 2 mg/kg p.o. s.i.d. during one week, followed by 1 mg/kg p.o. q 48h on a permanent basis and 12 g of a nutritional supplement Entero-Chronic® (Bioiberica S.A, Barcelona, Spain) p.o. s.i.d. The treatment led to weight gain and general health improvement.

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## CUTANEOUS MAST CELL TUMOURS IN A BENGAL TIGER (*PANTHERA TIGRIS*)

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A large variety of neoplasms have been reported in captive wild felids, however cutaneous mast cell tumours have rarely been described in exotic felids. A single case has been described in an Indian lion (*Panthera leo*). The present report describes the case of a 13 year old female Bengal tiger (*Panthera tigris*) at Parque das Ciencias Vigozoo, in Spain. Clinically, the animal presented hairless, occasionally with ulcerating, nodules, 1 to 15 cm in diameter on body and limbs. Samples of the skin tumours were fixed in formalin and embedded in paraffin wax for histopathological examination. Tumour was diagnosed as a histiocytic mast cell tumour with low mitotic activity, an atypical type with poorly-granulated cells. After this first surgical excision, a treatment was established with Metilprednisolona (Urbason<sup>®</sup> 40 mg, Sanfi-Avensis, S.A, Barelona, Spain) 2 mg/kg PO q24h one week, followed by 1 mg/kg q 48h PO on a permanent basis.

Unfortunately, six months after the first surgical excision, new masses were developed in the scapular area and on the limbs. These masses were also diagnosed as histiocytic mast cells tumours and a new treatment based on Lomustine (Cecenu<sup>®</sup> 40 mg, Medac, Hamburg, Germany) 60 - 90 mg/m<sup>3</sup> PO once a month was established.

Four months after initiation of treatment, concomitant disease unrelated to malignancy forced the euthanasia of the animal. Necropsy including sampling of the areas where tumours were previously located was performed. Histopathology did not reveal any signs of recurrence, suggesting that treatment with Lomustine (Cecenu<sup>®</sup> 40 mg, Medac, Hamburg, Germany) 60 – 90 mg/m<sup>3</sup> PO once a month, may be a valid treatment in the case of cutaneous mast cell tumours in species of exotic felids.

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## CLINICAL OUTCOME OF TWO DIFFERENT INTERNAL FIXATION METHODS OF A CLOSED COMMINUTED TIBIAL AND FIBULAR DIAPHYSEAL FRACTURE IN A WHITE BENGAL TIGER (*PANTHERA TIGRIS TIGRIS*)

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Fracture fixation in wild animals is challenging since coaptation splints and/or internal fixation will not be well tolerated. The following case report of a mature Bengal tiger (*Panthera tigris tigris*) (six years, 280 kg) with a closed comminuted, mid diaphyseal tibial and fibular fracture, outlines challenges related to internal fracture fixation in wildlife animals.

For primary fracture fixation a ten hole, 4.5 mm Buttress Plate with ten 4.5 mm screws on the medial aspect, and a nine hole 4.5 mm Dynamic Compression Plate with four 4.5 mm screws on the cranial aspect of the tibia were installed (Synthes<sup>®</sup>, Germany). Prior to plate fixation an intramedullary pin (Ø 7 x 130 mm) was brought into the tibial medullary cavity. After three Weeks the tibia and fibula refractured. For the revision surgery the tiger was referred to the IZW. A human T2 Interlocking tibia nail (Ø 10 x 270 mm, Stryker<sup>®</sup>, Germany) was implanted in a normograde fashion after removing all implants from the previous surgery. A patient-defined treatment plan was applied to ensure safe and effective immobilisation and intraoperative analgesia as well as postsurgical pain and wound management.

After approximately four Weeks of confinement and rest, the tiger was partially weightbearing on the operated leg. On control radiographs the proximal tibial fragment fractured and the proximal end of the interlocking nail and screws broke into the stifle joint. At this point the fracture of the diaphysis showed signs of secondary bone healing.

The tiger gained clinical and radiographic stability of the fractured tibia after additional weeks of rest and is using the leg in a functional pattern.

## ULTRASONOGRAPHIC EVALUATION OF ABDOMINAL ORGANS OF THE ASIATIC CHEETAH (*ACINONYX JUBATUS VENATICUS*); ONE OF THE MOST ENDANGERED FELIDAE MEMBERS

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### Summary

Ultrasonography of the only captive breed Asiatic cheetah (*Acinonyx jubatus venaticus*) housed at Pardisan rehabilitation center, Tehran- Iran, during routine annual check-up examination has been performed under general anaesthesia. Both of them were clinically healthy based on clinical examination and daily follow up. The ultrasonographic features of the liver, gall bladder, spleen, stomach, intestine, kidneys, urinary bladder, pancreas and adrenal glands were evaluated and measured in the present study. The ultrasonographic features were described subjectively and objectively. Asiatic cheetah that is known as “yuz” in Iran, is one of the most endangered members of the family Felidae in the world. So captive breeding and focus on health check-up planning should be necessary to save this species and this study can be used as a first step to establish future studies.

### Introduction

Abdominal organ diseases belong to the reasons that threaten the lives of cheetahs. The most common diseases in captive cheetahs are gastric ulceration, glomerulosclerosis, hepatic veno-occlusive disease (MUNSON *et al.*, 1999) and chronic renal disease (NICOLE *et al.*, 2005). Thus, we are presenting the normal ultrasonographic anatomy of the abdominal organs such as liver, spleen, gastro intestinal tract and urinary tract in Asiatic cheetah (*Acinonyx jubatus venaticus*).

### Material and methods

Two cheetahs were examined (1 male and 1 female, with 6 and 4 years old, 38 and 46 kg weight respectively) in this study. The Asiatic cheetahs housed at Pardisan wild life rehabilitation center in Tehran, Iran, were anaesthetised for annual examination checkup. Both of them were considered clinically healthy based on clinical examination and daily follow up. The ultrasonography has been performed after shaving the abdominal hairs and equipped by coupling gel. The ultrasonographic features of the liver, gall bladder, spleen, stomach, intestines, kidneys, urinary bladder, pancreas and adrenal glands were evaluated and measured. All the measurements were compared with African cheetahs and domestic cats references.

## Results and discussion

There were no significant differences between Asiatic cheetahs and domestic cats in subjective evaluation of abdominal organs. The splenic thickness at the location of the splenic vein was more than domestic cats' references although it was in the same range as African cheetahs (THRALL, 2013, ANN CARSTENS *et al.*, 2006). Hyper echoic well-defined nodular masses were detected within the splenic parenchyma in the male cheetah. The liver echogenicity was hypoechoic in comparison to the spleen and associated with well-marginated nodular masses in the liver parenchyma in the male cheetah. The gall bladder wall thickness was 0.17 cm in the female, although it was 0.46 cm in the male associated with hyper echoic gravity dependent sludge without acoustic shadow. Common bile duct diameter was 0.85 cm in the female and 0.78 cm in the male. The maximum wall thickness of the stomach was 0.41 cm in the female and 0.33 cm in the male. The intestine wall layering was the same as the stomach and the thickness was slightly more than in domestic cats (THRALL, 2013). Echogenicity of both kidneys' cortices was more than the one of the spleen with clear corticomedullary definition. The left and right kidney sizes were measured in length which were 6.46 cm and 5.54 cm in the left and 6.64 cm and 6.8 cm in the right kidney, in the male and female cheetah, respectively. The urinary bladder wall thickness measured 0.22 cm in the male and 0.19 cm in the female, which was thicker than in domestic cats (THRALL, 2013). The urinary bladder contained anechoic fluid in the female and small amounts of hyper echoic foci in the male. The pancreas was detected as a hypoechoic and pancreatic duct was clearly detectable within the pancreatic parenchyma. Both adrenal glands were visible as hypo echoic oval shape structures.

## Conclusion

The Asiatic cheetah (*Acinonyx jubatus venaticus*), known as "yuz" in Iran, is one of most endangered members of the family Felidae in the world, so captive breeding and planning focused on health checkup should be necessary to save this species. According to the author's knowledge, sonographic reference of abdominal organs is not existing in Asiatic cheetahs. Since there are only 38 living Asiatic cheetahs and we had access to two of them, this study could provide unique reference information and could be useful for the survival of this critical sub species. In addition, this study can be used as a first step to establish further studies.

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## MULTIPLE ORGAN FAILURE SECONDARY TO CHRONIC KIDNEY DISEASE IN A WILD EURASIAN OTTER (*LUTRA LUTRA*)

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One adult female wild Eurasian otter (*Lutra lutra*) weighing 3.4 kg, was found at a riverside under a bridge. It presented with severe depression and lethargy. While it was rescued and transported to the nearest rescue centre, the otter died. To find the exact cause of death, a necropsy was performed. When opening thoracic and abdominal cavities, severely dilated major cranial and caudal blood vessels running directly from the heart were observed. Also, dilated cardiomyopathy was found in right-side heart chambers. Histopathologically, most of the glomeruli in renal cortex were substituted by fibrotic tissues. Tubular necrosis was also examined. In the entire field of lung tissues, edema and ruptured alveoli were examined. Heart muscle fibers, especially in right-sided heart chamber were damaged, but fibrosis was not found. Both of liver and spleen showed severe congestion, and portal triads were dissociated. Taken together, the otter developed a severe multiple organ damage, which resulted in death likely due to high blood pressure resulting from chronic kidney disease. Characteristically, the pathologic lesion of the kidney was seen in the renal cortex, suggesting the possibility of adverse effect of toxic materials. Because of such suspicion, it is necessary to investigate the dead otter's natural habitat further. This subject is supported by Korea Ministry of Environment (MOE) as "Public Technology Program based on Environmental Policy (No. 2016000210002).

## VETERINARY WORK IN A SEAL REHABILITATION CENTRE: MOTHERLESS PUPS, LUNGWORMS AND OTHER DISEASES IN SEALS

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There are different marine mammal rehabilitation centres around the world that admit seals for rehabilitation. The patients are predominantly motherless pups, which would perish on their own; but also traumatised adult animals, which are injured due to being caught up in fishing nets or other human interference. Dealing with animals, especially wildlife and their various problems, makes every day a special day in a vet's working life.

Over the past six years, an increasing number of North Sea seals have been found suffering from lungworm infections, especially those in the tidal areas. The infection is predominantly affecting Common Seals (*Phoca vitulina*), but it is quickly spreading to Grey Seals (*Halichoerus grypus*) also. Lungworm infections are quite common in wildlife, but the number of severely infected animals has never been as great as it is at present. Two main species of worms, small and large, have been separated and identified as: *Otostrongylus circumlitus* and *Parafilaroides gymnurus*. This is a very serious and highly dangerous infection, which could have a devastating effect on the complete population of seals. Studies in Great Britain have revealed that even seals with a healthy weight have a very sensitive and delicate immune system. Overfishing, global warming, pollution of the seas and the environment – it all takes its toll.

## PERSISTENT PHOCINE DISTEMPER VIRUS (PDV) INFECTION IN TWO HARBOUR SEALS (*PHOCA VITULINA*) IN THE NETHERLANDS

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In 1988 and 2002, two phocine distemper virus (PDV) epizootics among harbour seals (*Phoca vitulina*) in northwestern Europe caused the death of tens of thousands seals. Severely affected animals died of acute necrotizing bronchointerstitial pneumonia. Rare, chronic persistent central nervous system (CNS) infections were reported in related morbilliviruses in dogs (canine distemper virus), dolphins (dolphin morbillivirus: DMV) and humans (measles virus). DMV was also reported in a harbour seal. Two adult male harbour seals stranded on the Dutch coast in 2001 (case 1) and 2014 (case 2). Based on counting dentin layers estimated age was 14 and 15 years, respectively. Significant lesions consisted of non-suppurative encephalitis in both animals, and spinal cord demyelination (case 1). Immunohistochemical staining with monoclonal CDV antibody demonstrated morbillivirus presence exclusively in the CNS, further confirmed by PCR. Viral sequence analysis of fusion protein (F), haemagglutinin (H), matrix (M) and phospho protein (P) genes demonstrated closest relations to PDV strains from previous local epizootics in 1988 (case 1) and 2002 (case 2). In both cases, viral sequences had distinct mutations in H and M genes. Attempts to isolate the virus in cell culture were unsuccessful.

Sequence data excluded DMV infection. As PDV was absent in the Northern Atlantic pick-up of PDV seems unlikely.

Seal age, histopathological findings and nucleotide sequence analyses suggested viral persistence of PDV/1988 and PDV/2002 strains in the CNS. These cases may be regarded as the first documented cases of persistent PDV infection in the CNS, similar to persistent infections of other morbilliviruses.

## SEXING GREATER FLAMINGOS (*PHOENICOPTERUS ROSEUS*): THREE TECHNIQUES IN COMPARISON

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Sexing animals in captive colonies has a crucial role in managing collections. DNA-based techniques are required to avoid errors in bird species with little sexual dimorphism. The aim of the study was to compare three molecular methods to sex greater flamingos. Blood from 42 birds, belonging to a private collection, was collected in EDTA tubes and DNA was extracted. Two PCRs, described by BALZIK *et al.* (2007) and by ITOH *et al.* (2001), were utilised. Amplification products were run on 2 % agarose gel and captured under UV light. Moreover, a modified version of the protocol described by Griffiths *et al.* (1998) was used: the P2 primer was labeled at the 5' end with a fluorophore (HEX) to detect the length of *chromo-helicase-DNA-binding* gene by capillary electrophoresis. Sizing of the fragments was carried out using ROX 500 size standard and GeneMapper v. 3.7 software (Applied Biosystems, USA). Sexing was successful with all techniques: the colony was composed of 18 males and 24 females. The Itoh's PCR gave an internal control fragment of approximately 185-bp and a female-specific fragment of approximately 160-bp. The Balzik's PCR resulted in a male or in a female band at approximately 590-bp or 330-bp, respectively. The Griffiths' modified PCR produced a single 373-bp fragment on males and two fragments (373-bp and 386-bp) on females. All three methods gave overlapping results. The Griffiths' technique, that was formerly considered inapplicable to greater flamingos, was able to clearly differentiate females from males, thanks to the high discriminative power of capillary electrophoresis.

## PRELIMINARY RESULTS OF AN EPIDEMIOLOGICAL STUDY ON THE ROLE OF REPTILES IN SALMONELLOSIS CASES: THE PIEDMONT REGION AS A MIRROR OF ITALY

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### Summary

Reptiles have become popular pet animals, raising the risk of reptile associated salmonellosis infection (RAS). The main aim of the present study was to estimate the impact of RAS on the population affected with salmonellosis in Piedmont, an Italian region. Two epidemiological studies were performed, by means of a telephone survey that was administered to every patient with salmonellosis. Between January 2015 and December 2016, 28 interviews were administered to patients afferent to the main Turin Hospital. Three cases reported the exposure to turtles. Between May 2016 and January 2017, 122 questionnaires were received from other medical centres. Eight cases reported the exposure to turtles. *Salmonella* was isolated from animals' or habitat samples in three cases. These preliminary data show that exposure to reptiles should always be considered as a risk factor in Italian salmonellosis cases, given also the ease with which people can come into contact with contaminated sources.

### Introduction

During the last decade, reptiles have become increasingly popular pet animals. However owners are not often aware of reptile-associated salmonellosis (RAS). *Salmonella* occurs naturally in the gastrointestinal tract of many reptiles as a part of their microbiota. Animals do not usually show clinical signs, but scatter the pathogen with faeces, infecting other individuals and contaminating their environment (GOUPIL *et al.*, 2012). In USA, RAS are regularly notified and represent the 6% of sporadic salmonellosis (HERNANDEZ *et al.*, 2012). Few information are available about the European (and Italian) situation, because a uniform system of notification is not currently in use (BERTRAND *et al.*, 2008). In particular, the Italian epidemiological situation could differ from the American one, since no ban on the sell of turtles with shell length less than 4 inches is enforced in Italy and public health campaigns have never been disseminated to prevent the transmission of the disease, The aim of the present study was to estimate the prevalence of RAS on the population affected with salmonellosis in Piedmont, an Italian region. Another goal was to evaluate the risk of exposure to *Salmonella* in public places across the region.

## Material and methods

To estimate the impact of RAS, two epidemiological studies were performed. The first on the population afferent to the Hospital "Città della Salute e della Scienza" of Turin: a telephone survey was administered to every patient with salmonellosis in order to characterize demographics, clinical severity, exposure to reptiles and other risk factors. Other risk factor considered were consumption of raw or undercooked eggs, meat, seafood, milk or dairy products, and direct/indirect contact with animals other than reptiles. Patients who reported reptile contact were asked questions about where the exposure occurred and, if the reptile was kept as a pet, about the animal husbandry. Patients owner of reptiles were asked to submit animals' and habitat samples in order to perform further analysis. Samples (oral and cloacal swabs, faeces and water) were always collected by a trained DVM. The study was then broadened to the entire Piedmont, thanks to the involvement of the local hygiene services: a question about reptile exposure was added to the salmonellosis investigation form. In case of a positive answer, the previously described survey was administered to the patient, if willing. To evaluate the risk of exposure to Salmonella in public places, observations of human visitors' and traders' behaviours, related to potential hygiene and pathogen transfer issues, were conducted. Three shops, three fairs and one temporary exhibition were visited, for a total of 400 minutes of observations. In details, visitors' and trades' hands were observed and the direct (reptile) or indirect (reptile habitat) contacts, and the subsequent touched items, were recorded. Moreover the presence of in situ public health warnings and safety protocols was assessed.

## Results and discussion

Between January 2015 and December 2016, 28 interviews were administered to patients of the Hospital "Città della Salute e della Scienza" of Turin. Eleven patients were females and 17 males, with age ranging between 10 months to 80 years. Three cases (9 %) reported the exposure to turtles. Patients were not able to define animals' species, other than declaring them "semiaquatic turtles". Two of them declared no risk factor other than having been in direct contact with turtles at relatives' house and by a lake, respectively. The third owned a turtle, but reported other risk factors and refused to submit specimens.

Between May 2016 and January 2017, local regional hygiene services sent 122 answers to the question about reptile exposure: eight (6.5 %) were affirmative. Of these, one person refused to further participate to the study and one patient reported the contact with a wild tortoise along with alimentary risk factors. The other six subjects owned pet turtles (*Trachemys scripta*) and agreed to submit samples. In one case, *S. Pomona* was isolated from turtles' cloacal swabs and water. Patient isolate was not available. In another case, *S. Typhimurium* I,4,[5],12:i:- was isolated from environmental samples: the PFGE patterns of these isolates and of the patient's one were identical. In a third case, further analyses are still on-going. The remaining three tested negative.

Preliminary results of the risk exposure evaluation were the followings. During the time frame, 61 contacts were reported, mainly indirect (58) and performed by visitors (54). The most touched item, after the contacts, was body/bag (30 times). Public health warnings and safety protocols were never noticed.

Although most cases of salmonellosis are caused by contaminated food consumption, infection acquired by contact with or exposure to carrier animals contributes to the disease burden. These preliminary data show that, also in Italy, exposure to reptiles should always be considered as a risk factor in salmonellosis cases. Even though some animals may test negative for Salmonella, they can not be excluded as cause of RAS due to intermittent shedding (MERMIN *et al.*, 2004). Moreover, to

discover the origin of salmonellosis, it is important to consider all possible circumstances when exposure to reptiles could have happened, not only the ownership. As delineated in the present case series, *Salmonella* may be acquired from wildlife or in every place where these animals are displayed. To prevent the disease, a comprehensive One Health approach is essential to disseminate good hygiene practice in reptile manipulation and ownership. From this point of view, zoos may have a pivotal educational role, since they can reach and, therefore, raise the awareness of a great number of people.

## Acknowledgements

We would like to thank the Italian Ministry of Health for funding (grant IZS PLV 02/13 RC).

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**THE FIRST THREE YEARS OF A NEW CONSERVATION BREEDING PROGRAMME FOR GREATER SAGE GROUSE (*CENTROCERCUS UROPHASIANUS UROPHASIANUS*): SUCCESSES AND CHALLENGES**

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Greater Sage Grouse have been listed as Endangered in Canada by the Committee on the Status of Endangered Animals in Canada (COSEWIC) since 1998 and have a Red List assessment of Near Threatened with population in decline by the International Union for Conservation of Nature (IUCN). This species exists at the northern edge of its range in the prairies of Alberta and Saskatchewan in Canada, and in this country its numbers have been reduced to less than 200 birds remaining in the wild. In 2014, the Calgary Zoo received the first 13 eggs from wild Sage Grouse to begin a captive breeding programme with a goal of producing chicks for reintroduction to the wild. To date a further 37 eggs have been received in 2015 and 2016. Incubation parameters were developed using information from standard galliform husbandry information and similar existing programmes for Atwater Prairie Chickens (*Tympanuchus cupido attwateri*) and Gunnison's Sage Grouse (*Centrocercus minimus*). Hatchability was 100 % in the first two years of the programme, and 92 % in 2016. Survival to 60 days of age was 66 % in captivity, compared with 25 % in the wild. Protocols were refined for chick management, nutrition, handling and general husbandry. All birds have been vaccinated for West Nile virus annually. Causes of morbidity and mortality have included trauma, bacterial infections, gastrointestinal impaction, perinatal issues, fungal infections and one case of predation.

## **TAENIA CRASSICEPS CYSTICERCOSIS IN A NILGIRI LANGUR (SEMNOPIITHECUS JOHNI)**

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An adult female captive born Nilgiri langur (*Semnopithecus johnii*) from Erfurt Zoo developed an edematous swelling of the left thigh, which persisted for several months and was associated with periods of depression and anorexia. The animal was finally euthanised due to a poor general condition and therapeutic resistance. A complete necropsy was performed and a broad spectrum of tissue samples were collected and prepared for histological examination. Samples from the mass of the left thigh were snap frozen for PCR analysis. At necropsy, the skeletal muscle of the left thigh was severely atrophic and replaced by a fluctuant multilocular cystic mass containing numerous sand grain sized whitish structures. Small cysts were also present in the left lung lobes and the left ventricular myocardium. Histologically, the cystic masses turned out to be metacestode tissue with evidence of numerous larval cestodes consistent with cysticerci. Cysticerci showed morphological characteristics of *Cysticercus longicollis*, the larval form of *Taenia crassiceps*, which was confirmed by PCR and sequencing of the mitochondrial cytochrome oxidase I gene. Non-human primates occasionally serve as aberrant intermediate hosts for several cestode species. So far, *Taenia crassiceps* cysticercosis has been described in a brown lemur and a ring-tailed lemur. This is the first case of a *Taenia crassiceps* cysticercosis in an Old World monkey species.

## DIAGNOSIS AND THERAPY OF ACUTE HIND LIMB PARALYSIS IN A EUROPEAN BROWN BEAR (*URSUS ARCTOS ARCTOS*)

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A 23-year-old female European brown bear (*Ursus arctos arctos*) from the Bärenwäld in Anholt, Germany showed signs of acute hind limb paralysis (paraplegia) with intact nociception. A thoracolumbar neurolocalisation was suspected due to upper motor neuron signs of the hind limbs and no neurological signs to the fore limbs (though cervical lesions were not excluded). Main differential diagnoses were intervertebral disc disease and vertebral (sub)luxation or (pathological) vertebral fracture among others. The brown bear was initially treated with tramadol twice daily 2 mg/kg, diazepam twice daily 0.5 mg/kg and prednisolon once daily 0.5 mg/kg. After one week, lateral radiographs of the entire vertebral column were made as well as ventrodorsal pelvic radiographs. Only the vertebral column revealed lesions, consistent with spondylosis of thoracic vertebrae without clinical importance. Blood values were within normal limits and the bear had a good appetite. Computed tomographic (CT) imaging of the entire vertebral column showed a fracture of the corpus of T14. A conservative approach was chosen, consisting mainly of confinement to a small enclosure. After six weeks, the bear gradually started to move both hind limbs and after three months she was able to put her hind limbs under her back. This progress continued gradually. In October 2016, she could walk for about 5 to 10 meters consecutively. Up until her hibernation period in December, the bear was gradually tapered of the prednisone and tramadol. She had her regular hibernation period from December 2016 until March 2017 and showed further improvement thereafter. This case report supports the additional value of CT imaging in addition to radiographs in Ursidae and shows that conservative management of vertebral fractures in these species is a feasible option in certain cases.

## RETROSPECTIVE STUDY OF MORTALITY ASSOCIATED WITH GENERAL ANAESTHESIA IN CHEETAHS (*ACINONYX JUBATUS*)

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Mortality rates during immobilisation, anaesthesia and translocation is a concern for the cheetah (*Acinonyx jubatus*) as 21 % death has been reported since the inception of the Cheetah Metapopulation Project translocations in 2011. These translocations ensure the genetic health of the South African metapopulation but the continued loss of animals threatens its viability. The requirements for research on preferred immobilisation techniques that will minimise the loss of cheetahs is essential, however reports on the suitability of anaesthetic drugs for immobilisation and anaesthesia are very limited. In this study, a questionnaire was sent to all zoo and wildlife veterinarians familiar with cheetah immobilisation in order to evaluate the potential causes of this high mortality rate. Individual characteristics, anaesthesia protocols, monitoring parameters as well as histopathological results obtained during post-mortem examinations were analyzed. Although systemic arterial hypertension, transient seizures, metabolic acidosis and hypoxia are commonly reported during these procedures, stress and hyperthermia appear to be preponderant factors in the high mortality rate independently of the anaesthetic protocol and the capture method. The first results suggest between 10 and 20 % of anaesthetised individuals with hyperthermia, some of them reaching a rectal temperature as high as 43°C. The association between behavioural stress scores, rectal temperatures and the successful management of the felids with post-induction hyperthermia were also discussed. This retrospective study represents the first overview of captive and free-ranging cheetah mortality during anaesthesia.

## RELATIONSHIP AMONG CORTISOL, TESTOSTERONE AND DEHYDROEPIANDROSTERONE LEVELS DETECTED IN FAECES OF SILVERBACK GORILLAS

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The aim of this study was to determine the relationship between cortisol, testosterone (T) and dehydroepiandrosterone (DHEA), which are implicated in stress responses in mammals, in Gorilla (*Gorilla gorilla gorilla*) faeces. Faeces from three silverback gorillas (M1: 28 years old in a group with 2 adult females and 2 offspring; M2: 19 years old in a group with 6 adult females and 7 offspring; and M3: 23 years old in a group with 2 adult females and 1 offspring) held in different zoos were sampled for a month. A mean of 20 samples per individual were non-invasively collected. After hormone extraction using methanol, cortisol, T and DHEA were analysed by enzyme immunoassay (EIA). The analysis of variance and post-hoc analysis results showed that the male housed in the most crowded group (M2) had statistically higher cortisol levels than M1 and M3. In addition, M3 had lower T concentrations compared to the other two male silverbacks. No differences were observed in DHEA levels among the three males. However, a significant positive Pearson correlation was observed between T and both cortisol and DHEA levels. Finally, a tendency to positive correlation was observed between DHEA and cortisol concentrations. Higher cortisol levels detected in M2 might reflect social stress due to higher frequency of interactions among individuals. The positive correlation found between both hormones supports the hypothesis that maintaining their social rank and the group stability comes at a cost. Results of this study demonstrate for the first time that DHEA can be measured in gorilla faeces by EIA. The results of this study show the complex interaction among the hormones assessed. Improving our knowledge on the complex interplay among steroid hormones can provide a powerful tool for studying the entangled stress responses.

## CONTRASTING *MYCOBACTERIUM BOVIS* IN FREE-RANGING CERVIDS IN THE GREAT LAKES STATES OF MINNESOTA, MICHIGAN AND INDIANA, USA

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Michigan identified *Mycobacterium bovis* (bTB) as a significant issue in captive cervids, free-ranging white-tailed deer (*Odocoileus virginianus*), cattle, and peridomestic mammals since 1994, with the introduction occurring possibly as early as 1975. Michigan was declared bTB free in 1979, but lost this status in 2000 after cattle became infected with wild deer serving as a maintenance host for bTB. Minnesota identified bTB in a northern cattle herd in 2005 and sampling found bTB in local wild deer. Minnesota returned to bTB free status in 2011, eradicating bTB in both cattle and deer. Meanwhile, Indiana had been bTB free since 1984, but in late 2008 and early 2009, a single cow and a captive cervid herd were detected as infected through slaughter surveillance in southern Indiana. In 2010, an additional premise was identified as bTB positive in this same area. All cases were ultimately linked by whole genome sequencing (WGS). In May 2016, two additional premises within 4 miles of the 2009 cervid farm were detected through cattle sent to slaughter. A testing protocol involving wild deer and susceptible farms was implemented. A total of 16 targeted and 938 hunter-harvested deer were tested within a 10-mile radius. One positive deer and a raccoon (*Procyon lotor*) were found in the targeted sample from one affected farm. Results of WGS indicated that the directionality of the infection was from the affected farm to the wild deer.

## SUCCESSFUL TREATMENT OF SYSTEMIC LUPUS ERYTHEMATOSUS IN A MONGOOSE LEMUR (*EULEMUR MONGOZ*)

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A twelve-year-old obese female mongoose lemur (*Eulemur mongoz*) presented depigmentation of the nose and alopecia of the cutaneous-mucosal junctions around the eyes, mouth and nose evolving over nine years.

Histopathological and immunohistochemical exams of skin biopsies revealed an epidermal and follicular interface dermatitis with lymphocytic infiltration, consistent with discoid lupus erythematosus (DLE).

Circulating anti-nuclear antibodies (ANA) were detected prompting the search of lesions of systemic of lupus erythematosus (SLE), as usually found in humans. Glomerulonephritis associated with proteinuria but no blood biochemistry anomalies were found. No other lesion of SLE was detected.

Methotrexate (Méthotrexate 25 mg/ml®, Biodim, Paris, France) was used at a dose of 5 mg/kg subcutaneously once a week. No adverse effect was observed. Clinical healing was seen after two months with hair regrowth and pigmentation of the nose. After three months, histopathological examination of new biopsies confirmed that the skin was close to normal with no more lymphocytic cytotoxicity. Dosage of ANA is currently in progress.

The animal additionally had ovarian cysts and high levels of sexual hormones. Ovariohysterectomy was performed as SLE can be exacerbated by estrogens.

In human medicine, methotrexate is not the first therapy of choice for SLE. Corticoids were avoided as this animal had minimal hepatitis. All galenic forms of doxycycline and cyclosporin were refused by the animal. Methotrexate has the advantage of being administered once a week without any adverse effect at this dose in humans, dogs, and horses. The animal will be checked regularly for long-term effects of this molecule.

## PROSTATE TUMOR INDUCED AZOTEMIA AND SECONDARY GAS-FORMING BACTERIAL INFECTION IN A WILD BOAR (*SUS SCROFA*)

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One male wild boar (*Sus scrofa*) appeared in the hiking trail of the Bukhansan National Park, which was fully grown weighing 200 kg, panting severely, and bleeding from nose, mouth and anus. During the rescue, the dead wild boar was transferred to the Pathology Laboratory for autopsy. When thoracic and peritoneal cavities opened, the ammoniac odor came up, which is characteristic of the patient with azotemia. Afterwards, the huge bulged bladder caught the eyes; there was a massive prostate tumor around the prostate urethra. The volume of urine reached 1.75 L. The prostate tumor appeared to be an active working gland producing a large amount of secretions.

Morphologically, the tumor is "acinar type" where tumor is thought to arise from or recapitulate prostatic acini. The acinar type (usual type) is characterised by back-to-back proliferation of small to intermediate sized tumor acini with scant to moderate intervening stroma.

Blood was not clotted, all organs were necrotic and the texture was like sponges containing gas, which was presumably an infection by gas-forming bacteria. The bacterium was identified *Clostridium novyi* after microbiology tests; bacterial culture (aerobic/anaerobic) and PCR. The etiology is presumed to be the azotemia caused by the prostate tumor, which weakened blood vessels and organs, resulting in secondary infection. To the best of the authors' knowledge, this is the first description of the prostate tumor induced azotemia and a secondary bacterial infection resulting in severe internal organ malfunction and death in a wild boar in Korea. This subject is supported by Korea Ministry of Environment (MOE) as "Public Technology Program based on Environmental Policy (No. E416-00021-0602-0).

## CHOLANGIOCARCINOMA WITH MULTIPLE ORGAN METASTASIS IN A CAPTIVE PUMA (*PUMA CONCOLAR*)

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A 17-year-old captive male puma (*Felis concolor*) died in the Grand Park Zoo, Seoul, Republic of Korea. The animal was in good body condition and no clinical symptoms had been observed prior to death. At necropsy, one of the liver lobes was severely involved carrying one big mass, 100 mm in diameter and small nodules were disseminated throughout the liver, which were 5 to 40 mm in diameter tawny to white nodules were scattered in the lung, stomach, kidney, heart and diaphragm. The mass in the liver had an umbilicated appearance. Palpation of the nodules revealed a firm texture due to abundance of connective tissue. This firm texture distinguishes a cholangiocarcinoma from a hepatocellular carcinoma which generally have soft and friable texture. The gall bladder was empty and atrophied while the spleen was obviously enlarged. Histopathologically, the mass and nodules were composed of tumour cells arranged in typical papillary and acinar formation. Mitotic figures were moderate to abundant. Immunohistochemical examination revealed the cells to be strong positive for cytokeratin, moderate positive for CD 10 and negative for carcinoembryonic antigen, fetoprotein and hepatocyte paraffin-1. Taken together, the tumour was diagnosed as a rare case of cholangiocarcinoma having papillary type in a puma. To the best of the authors' knowledge, this is the first description of the cholangiocarcinoma (bile duct carcinoma) in a captive puma (*Felis concolor*) in Korea. This subject is supported by Korea Ministry of Environment (MOE) as "Public Technology Program based on Environmental Policy (No. E416-00021-0602-0).

## A GOOD TIME TO DIE? INVESTIGATING THE SEASONALITY OF MORTALITY IN ZOO ANIMALS

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Seasonal differences in adult mortality are known in humans and domestic animals, and have also been investigated in free-ranging primates (GOGARTEN *et al.*, 2012). By contrast, studies on seasonal mortality patterns in zoos are rare (GULL *et al.*, 2010). We developed a method to quantify the degree of seasonality in mortality data. Mortality in a population is expressed on a monthly basis. The proportion of mortality in the month with the lowest mortality is considered the 'baseline mortality', deducted from the mortality of each month, and the remainder is defined as 'above-baseline mortality'. Subsequently, the proportion of this 'above-baseline mortality' that is represented by the three consecutive months with the highest 'above-baseline mortalities' is calculated and defined as 3MM. 3MM can vary between 0 (mortality is equal in all months) or 1 (all above-baseline mortality occurs within 3 consecutive months). We illustrate the concept with examples from two groups of zoo mammals. In ruminants, seasonal mortality mainly occurs in winter, but there is no indication of a higher susceptibility for species originating from warmer climates to winter deaths; rather, 3MM is linked to reproductive events and is higher in seasonal breeders. Differences in gender-specific 3MM patterns occur especially between cervids and caprids. In carnivores, there is also some concentration of winter mortality, but pinnipeds display higher mortality in summer. Correlations of relative life expectancy (MÜLLER *et al.*, 2011) with 3MM did not yield significant results so far, indicating that the observed patterns are not linked to overall husbandry success.

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## NEOPLASIA IN ZOO AND FREE RANGING ANIMALS IN AUSTRIA: A RETROSPECTIVE STUDY

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This survey presents retrospective data on neoplasia in zoo animals (ZA) and free ranging animals (FRA) in Austria. We analysed pathology reports from 01/2010 to 12/2016 performed at our pathology lab.

122 tumours in 117 animals from 68 species are documented. 34 different types of tumours in ZA and FRA were detected, minimum one neoplasia in every class of vertebrates. Tumours were found in 88 mammals (58 ZA/30 FRA), 19 birds (18 ZA/1 FRA), 8 reptiles (8 ZA) and one each in amphibian and fish (both ZA). We found more neoplasms in ZA (73.5 %) than in FRA (26.5 %).

In ZA adenomas and adenocarcinomas of the liver were often noticed. The artiodactyla was the order with most frequent tumours (17 animals with leiomyoma, adenoma and haemangiosarcoma). In FRA the integument was often affected; mostly fibromas from roe deer (*Capreolus capreolus*) and red deer (*Cervus elaphus*). 46.8 % of the tumours turned out to have been unpublished in the species where they were found. Such as a seminoma in a Capercaillie (*Tetrao urogallus*) and a leiomyosarcoma in an Eurasian otter (*Lutra lutra*).

The higher tumour prevalence in ZA (5.69 %) in contrast to FRA (0.78 %) may result from the health monitoring of ZA compared to FRA, but could also indicate anthropogenic influences such as husbandry and increase in life span. This study gives first insights into the topic of cancerogenesis. An in depth investigation into causes and mechanisms of tumour progression and the impact on different species would shed light into this complex topic.

## COPPER ISOTOPES AS A PROMISING DIAGNOSTIC TOOL FOR NEOPLASIA IN CAPTIVE WILDLIFE

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Copper isotope <sup>65</sup>Cu and <sup>63</sup>Cu are used as biomarkers for neoplasia in humans and domestic animal since three years. Neoplastic cells tend to turn their metabolism to glycolysis leading to an increase in lactate within the affected tissue. This process is called the Warburg effect. The heavy isotope <sup>65</sup>Cu is known to form a more stable bond with lactate, compare to the light isotope <sup>63</sup>Cu. Because of this difference in bonding capacity the ratio of these two isotopes changes in blood. The difference between isotopes called delta Cu (dCu) is measurable by multiple-collector inductively coupled plasma mass spectrometry (MC-ICP-MS). This new diagnostic tool validated in human and pet oncology present interesting results both in terms of diagnostic and follow-up of neoplasia.

As neoplasia in captive wildlife are more and more a veterinary concern, trial and development of new diagnostic tools are necessary. Then the technique used in pet oncology has been tested in 33 serum samples from 14 different species in order to assess the interest and the feasibility of dCu measurement.

A significant variation of the dCu from species to species is noticed, ranging between 0.2 and -1. It goes the same at the intraspecific level, showing a strong individual factor. However for the same healthy animal the value of the dCu is stable over time with slight variation between 0.1 to 0.2.

Finally the difference between a healthy and an animal with neoplasia show the same trend for dCu variation compared to pet samples with a decrease of the marker (> 0.5) when there is a neoplasia. This preliminary study shows interesting and promising outcomes and tends to validate the use of copper isotopes as relevant biomarkers in wildlife.

**A DOUBLE-BLINDED, RANDOMISED COMPARISON OF MEDETOMIDINE-TILETAMINE-ZOLAZEPAM AND DEXMEDETOMIDINE-TILETAMINE-ZOLAZEPAM ANAESTHESIA IN FREE-RANGING BROWN BEARS (*URSUS ARCTOS*)**

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Dexmedetomidine-tiletamine-zolazepam (DTZ) has been suggested to offer advantages over medetomidine-tiletamine-zolazepam (MTZ) in the anaesthesia of bears. We compared anaesthetic features, blood parameters, and physiological responses to either medetomidine-tiletamine-zolazepam or dexmedetomidine-tiletamine-zolazepam using a double-blinded, randomised experimental design during 40 anaesthetic events of free-ranging brown bears (*Ursus arctos*) either captured in Sweden or in Canada. In Sweden, bears allocated to the MTZ group received an average dose of 93.62 µg/kg of medetomidine and 4.69 mg/kg of tiletamine-zolazepam; those allocated to the DTZ group received 57.51 µg/kg of dexmedetomidine and 4.87 mg/kg of tiletamine-zolazepam. In Canada, we used 52.23 µg/kg of medetomidine and 2.5 mg/kg of tiletamine-zolazepam or 21.97 µg/kg of dexmedetomidine and 1.6 mg/kg of tiletamine-zolazepam. Induction time, the need for supplemental drugs, and capture-related stress were analysed using generalised linear models, but anaesthetic protocol did not differentially affect these variables. Arterial blood gases and acid-base status, and physiological responses were examined using linear mixed models. We documented acidemia (pH of arterial blood < 7.35), hypoxemia (partial pressure of arterial oxygen < 80 mmHg), and hypercapnia (partial pressure of arterial carbon dioxide ≥ 45 mmHg) with both protocols. The partial pressure of arterial carbon dioxide increased as respiratory rate increased with MTZ ( $p < 0.05$ ), but not with DTZ, demonstrating a differential drug effect. Differences in heart rate, respiratory rate, and rectal temperature among bears could not be attributed to the anaesthetic protocol. Overall, we did not find any benefit of using dexmedetomidine-tiletamine-zolazepam instead of medetomidine-tiletamine-zolazepam in the anaesthesia of brown bears.

## VERTEBRAL VENTRAL SUBLUXATION STABILISATION WITH BILATERAL DYNAMIC COMPRESSION PLATE IN PERSIAN LEOPARD: THE FIRST CASE REPORT WITH NEUROLOGICAL IMPROVEMENT

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A five-year-old female Persian leopard was presented with pelvic limbs plegia and laceration in paralumbar area following entrapment. The clinical signs revealed lethargy, loss of appetite and body-weight, muscle atrophy of pelvic limbs, lateral recumbency and paraplegia. The radiological examination revealed ventral subluxation and disc space narrowing of the lumbar 4-5 intervertebral articulation with irregular periosteal productive response involving the endplates and central aspects of the vertebral bodies. There were also a few small air pockets in the adjacent sublumbar retroperitoneal space in order to an open wound at right flank. Hematological and biochemical analysis showed leukocytosis and uremia due to open wound and dehydration. Anaesthesia was induced with Medetomidine (1000 µg/m<sup>2</sup>, Provet, Istanbul-Turkey) and Ketamine HCl (20 mg/kg, Alfasan, Woerden-Holland) intramuscularly by blowpipe and then was continued with Propofol (4 mg/kg, Braun, Hessen-Germany), intravenously. After intubation, anaesthesia was maintained by Isoflurane. Spinal stabilisation and reduction was performed with double DCP 3.5 mm through dorsolateral approach. Open wound was managed by indwelling of drain. The Persian leopard tolerated surgery and the drain well. Antibiotic therapy with Amoxicillin clavulanate (25 mg/kg, orally, Farabi, Isfahan-Iran) was administrated for 10 days. Pain management with Meloxicam (0.2 mg/kg, orally, Razak, Tehran-Iran) for three days was administered. The leopard began showing clinical improvement and weight-bearing on day five postoperatively. The paraplegia improved four weeks after the surgery and she walked and jumped normally with no sign of nerve weakness. This report shows the successful treatment of spinal column injury and the improvement of neurogenic deficits in a Persian Leopard in Iran.

## **UNDERSTANDING IMMUNITY AGAINST FATAL ELEPHANT ENDOTHELIOTROPIC HERPESVIRUS (EEHV) INFECTION**

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Elephant Endotheliotropic Herpesvirus has claimed the life of nearly one-third of all Asian elephant calves' captive born in Europe since January 1, 1985. However, there are specific captive breeding facilities that are highly affected by EEHV haemorrhagic disease (EEHV-HD), and others, which never experienced a fatal case, even with lethal virus present in the herd.

EEHV related disease presents itself as an acute haemorrhagic syndrome due to a vast endothelial destruction, usually causing sudden death. Therefore, we studied a coagulation factor (Factor VII), an important component of the coagulation cascade, in which its deficiency may cause bleeding and increased coagulation times. We investigated the presence of point mutations in this gene in four EEHV-HD European fatal cases, and preliminary results show a highly similar genetic pattern for these individuals. The gene is composed by eight exons. After amplification and sequencing we found that exon 1 presented one single nucleotide polymorphism (SNP); three out of the four individuals presented identical sequences (G), and one animal showed a heterozygous position (C/G). Another finding was in exon 6, where three individual presented polymorphism (C/G) and one individual is homozygote for this position (only G). No other heterozygosis was detected for the other exons.

Establishing a causative relation between the presences of coagulation factor VII mutations with EEHV-HD is not yet possible, but we believe that this study will allow in a near future an understanding if this genetic factor is an important element on the onset of the viral disease.

## CHRONIC ZINC TOXICOSIS IN A MIXED GROUP OF PSITTACINE BIRDS: DIAGNOSIS AND MANAGEMENT IN A COMPLEX CLINICAL CONTEXT

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Twenty recurrent episodes of apathy, dysorexia and regurgitations were observed in eight individuals of three psittacine species - galah (*Eolophus roseicapilla*), burrowing parrot (*Cyanoliseus patagonus*) and sun conure (*Aratinga solstitialis*) - at ZooParc de Beauval over a one-year period. These birds were part of the bird show and were subject to frequent aviary transfers. Clinical improvement was first observed after 2 days of hospitalisation in a warm room with medical treatment (fluid therapy, metoclopramide, vitamins B1, B2, B6, B12, E and selenium) although no etiologic diagnosis was made. Chronic zinc toxicosis was suspected based on recurrence of symptoms 48h to 72h after transfer into galvanised wire cage and after other potential causes were ruled out by complementary exams, (bloodwork, coproscopy, radiography and endoscopy). Diagnosis was confirmed in two galahs with ante-mortem blood dosage (> 294 µg/dL) and post-mortem liver dosage (180 µg/g liver and 299 µg/g liver dry weight) and excluded in one burrowing parrot (86 µg/g liver dry weight). Post-mortem histologic changes were unspecific but compatible with zinc toxicosis in three cases (hepatic hemosiderosis (2/5), renal tubular degeneration (3/5)). The remaining birds (two sun conures) were changed from environment and isolated for several weeks. Substrate, location and aviary were reintroduced one after the other over a 6 week-period (two weeks for each new element). No new episodes were recorded. The aviaries galvanisation (carried out at the zoo) is suspected to have caused this toxicosis although it remains uncertain.

This case series illustrates the challenge of low prevalence chronic zinc toxicosis diagnosis in a mixed group of birds housed in multiple aviaries. Species sensitivity is highlighted in an unspecific clinical context, where only two cases have been confirmed.

## METHOD FOR THE DETECTION OF *MYCOBACTERIUM AVIUM* SUBSP. *PARATUBERCULOSIS* DNA IN FAECAL AND ENVIRONMENTAL LARGE VOLUME SAMPLES

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Paratuberculosis in cattle, which is widespread in German herds, is caused by *Mycobacterium avium* subsp. *paratuberculosis*. The disease has a significant economic effect and results in substantial losses in livestock, wildlife and zoological specimens. The detection of mycobacteria by culture or molecular methods and the evaluation of histopathological lesions constitute the most effective methods for confirming a diagnosis of paratuberculosis, but the risk of false-negative results remains. In this study, a DNA extraction method was developed, based on magnetic capture (MC), which allows the investigation of a large sample masses (5 g versus a few milligrams) to increase the probability of *MAP* DNA detection in faecal samples as an intravital test and in soil samples as an environmental test. One hundred forty-eight faecal samples from 21 African pygmy goats (n = 100), from one snow goat (n = 8) and eight java-banteng (n = 40, negative control group) were screened for *MAP* DNA with the established MC DNA extraction method and a following species specific real-time PCR. In addition, 30 soil samples from the snow goat enclosure (n = 10, positive sample pool), from the java-banteng enclosure (n = 10, unknown sample pool) and from purchased terrarium soil (n = 10, negative sample pool) were also analysed. The extraction methods yield reliable results by the real-time PCR analysis. The newly developed method could be a useful tool for the detection of *MAP* intra vitam in animals and in their environment. This could lead to improved enclosure sanitation measures to further prevent the infection of valuable livestock with *MAP*.

## COINCIDENCE OF CUTANEOUS DEMODICOSIS AND UV-INDUCED SKIN NEOPLASIA IN TWO GOELDI'S MARMOSETS (*CALLIMICO GOELDII*)

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Two Goeldi's marmosets (*Callimico goeldii*) from the same husbandry consecutively showed multiple progressive alopecic to ulcerating skin lesions at the lower abdomen and groin. First, the female developed severe changes, leading to euthanasia due to poor prognosis, and went to necropsy. Histologically, skin lesions revealed large quantities of parasitic organisms consistent with *Demodex* spp. within infundibular hair follicles, together with moderate mononuclear inflammation and irregular acanthosis, actinic keratosis, carcinoma *in situ* and squamous cell carcinoma of varying degree. A few years later, the male marmoset showed similar skin alterations. Microscopy of skin scrapings and biopsies also revealed abundant *Demodex* spp., associated with moderate inflammation and hyperplastic to dysplastic epithelial changes. Despite repeated anti-parasitic treatment (0,1 ml Advocate® Spot on, 0,3 mg/kg Ivermectin p.o., 2 mg Simparica p.o.) the lesions did not improve. Due to general deterioration, this marmoset was also euthanised and sent to necropsy. Although no demodex organisms were detected at this point, the skin lesions histologically resembled those of the other.

The epidermal changes were most likely induced by ultraviolet radiation. Both animals had three times daily access for 30 minutes to an UV-lamp (Osram Ultra Vitalux 300W) and frequently hung very close to it. An occasional coincidence of mite infection and cutaneous epidermal neoplasia is also described in humans and cats. This is probably due to a preferred parasitic settlement in immunologically impaired (pre-) neoplastic tissues. However, parasite induced chronic inflammation may also contribute to tumor genesis. In either case, an appropriate UV regime for captive marmosets should be reconsidered.

## RETROSPECTIVE STUDY OF NEOPLASMS IN CAPTIVE AFRICAN HUNTING DOGS (*LYCAON PICTUS*) IN FRENCH ZOOLOGICAL INSTITUTIONS

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It is often reported that African hunting dogs (*Lycaon pictus*) are prone to develop tumours but no study proves this. We tried to investigate this hypothesis through a survey. A questionnaire was sent to all the French zoological institutions that have once held African hunting dogs as reported by Species 360. All eleven French holders responded to the survey.

The studied population is composed of 217 individuals of more than one year old from 1980 to date; with almost an equal sex ratio (109 males, 106 females and 2 unknown sex). A total of 18 individuals from 5 parks presented 23 tumour cases; one case being defined as the existence of one type of neoplasm assessed by histology in one individual.

Tumours are distributed in 17 different histological tumours as follows: 6 malignant cutaneous tumours (5 apocrine sweat gland adenocarcinomas, 1 basal cell carcinoma), 5 benign cutaneous tumours (1 acrochordon, 1 apocrine sweat gland adenoma, 1 well differentiated mastocytoma, 1 sebaceous adenoma, 1 trichoblastoma), 4 adenocarcinomas (2 mammary adenocarcinomas, 1 anal sac adenocarcinoma, 1 nasal adenocarcinoma), 2 hemangiosarcomas, 1 abdominal anaplastic carcinoma, 1 epulis, 1 diffuse histiocytic sarcoma, 1 insulinoma, 1 leiomyoma and 1 osteosarcoma.

Neoplasms were diagnosed at necropsy for 12 of the 18 individuals. The average age at diagnosis is 9.58 years. A trend to gender predisposition is supposed as 67 % of the individuals are females.

Data are being collected from the EEP members for further investigations and try to find predisposition factors such as a genetic origin.

## LEAD INTOXICATION IN FREE RANGING WHITE-TAILED EAGLES (*HALIAEETUS ALBICILLA*)

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Lead intoxication is a well-known problem in free ranging white-tailed eagles (*Haliaeetus albicilla*) in Germany and elsewhere, however clinical symptoms, prognosis and outcome of a large number of intoxicated birds has not been reported in the literature. (MÜLLER *et al.*, 2007). The aim of this study was to analyse data of free ranging white-tailed eagles presented to at the Small Animal Clinic of the Freie Universität Berlin.

In this retro- and prospective study, data from 210 free ranging white-tailed sea eagles were included. Clinical examination, haematological and blood chemical parameters, as well as blood lead concentrations, diagnostic imaging and outcome were evaluated. Blood lead concentrations > 0.4 ppm were considered consistent with lead intoxication (MÜLLER *et al.*, 2007).

Lead intoxication was diagnosed in 90 of 210 white-tailed eagles (43 %). Common clinical symptoms included apathy (73 %), dyspnoea (49 %), hemolysis (47 %), biliverdinuria (42 %), cachexia (28 %), vomiting (23 %), and crop stasis (21 %). The median blood lead concentration was 2.4 ppm (range: 0.4-21.7 ppm). Radiographically metal-dense structures within the gastrointestinal tract were found in 34 (38 %) eagles. Thirty-nine animals (43 %) died from consequences of lead intoxication. Nineteen individuals (21 %) were euthanised due to other diagnostic findings. Thirteen eagles (14 %) were euthanised due to their poor general condition associated with lead intoxication, while 22 (22 %) eagles recovered after therapy.

Due to non-specific clinical symptoms and high incidence every white-tailed eagle found injured should be checked for lead intoxication.

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## INVESTIGATIONS ON THE FEATHER MITES OF THE NORTHERN LAPWING, *VANELLUS VANELLUS*

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### Summary

The plumage of 75 previously frozen northern lapwings (*Vanellus vanellus*) from Central Germany was examined for the presence of ectoparasites. Of these, 99 % were infested with 1 to 154 feather mites. From the 75 northern lapwings, 1003 single feather samples were examined via stereomicroscopy for feather mites; adult specimens were counted, removed from the feathers, and mounted in Berlese-mixture for further evaluation. Out of 803 positive feathers, 705 mites could be evaluated. The majority (99 %) were identified as *Triphyllochaeta vanelli* (Pterolichidae: Magimeliinae) whereas only 1 % belonged to the species, *Leptosphyra centropoda* (Xolalgidae: Ingrassinae). No evidence of feather damage was detected. The findings in this study concur with previous descriptions of the feather mite fauna of *Vanellus vanellus* and give further information about the intensity and locality of infestation. They also point at feather mites having a rather low negative effect on their hosts.

### Introduction

The northern lapwing, *Vanellus vanellus* Linnaeus 1758, a migratory bird of the family Charadriidae, is mainly native to Mediterranean areas of Europe and Asia. Lapwings prefer resting and breeding in areas of low vegetation and frequently settle in agricultural areas (KOOIKER and BUCKOW, 1997).

The northern lapwing is currently considered as near threatened (IUCN 2016). Its status in Germany is among the strictly protected species (§ 1 Appendix 1 BArtSchV 2005). Investigations on feather mites of *Vanellus vanellus*, their prevalence, and intensity of infestation is therefore often reduced to examining single feathers, found by chance or taken from birds held in captivity.

To date only two feather mite species from the plumage of the northern lapwing in Germany have been described. *Triphyllochaeta vanelli* Canestrini, 1878 (Pterolichidae: Magimeliinae) seems to be strictly adapted to this one host species (SCHÖNE and SCHMÄSCHKE, 2015; SCHMÄSCHKE *et al.*, 2004; MIRONOV *et al.*, 2002), while *Leptosphyra centropoda* Mégnin 1877 (Xolalgidae: Ingrassiinae) has been reported from other charadriiform species as well (PEDROSO *et al.*, 2015; DABERT, 2000; GAUD and ATYEO, 1981; GAUD, 1972).

### Material and methods

Seventy-five northern lapwings were examined for the occurrence of ectoparasites. The birds had been injured in a hailstorm and had to be euthanised due to animal welfare concerns (ROESSLER, 2011). Until parasitological examination, birds were frozen at -20°C, then thawed for at least 12 hours.

Every bird was macroscopically examined for ectoparasites. Three feather samples were taken each from the tail feathers, the primaries and the secondaries of each wing since, according to SCHÖNE and SCHMÄSCHKE (2015), feather mites prefer primal feathers. In case of contamination with dirt or blood, feathers were not examined.

Every sample was examined via stereo microscopy (magnification of 1:25 to 1:40); feather quills were surveyed for lesions or abnormalities and opened for microscopic examination at random. All visible mites, independent from their stage of development, were counted on each feather. Samples from each infested feather were taken and mounted in Berlese-mixture for further evaluation. Up to five adult mites from each sampled feather were evaluated via light microscopy. The identification and taxonomical integration follows GAUD and ATYEO (1996) and MIRONOV *et al.* (2002).

## Results and discussion

At least nine feather samples were taken from every bird. Feather mites were found on 74 northern lapwings (99 %). Of the 1003 feathers that were examined, 803 (80 %) were positive for feather mites. The intensity varied between 1 and 154 counted mites per feather (figure 1).

Adult mites mounted in Berlese-mixture were measured and evaluated. Of 705 mites, 701 (99 %) were identified as *Triphyllochaeta vanelli* (figure 2, 3); four mites (1 %) were assigned to the species *Leptosphyra centropoda* (figure 4). No quill mites were discovered in this study.

The results of this study confirm earlier studies on the feather mite fauna of the northern lapwing and on the distinctive host specificity of most feather mites (GAUD and ATYEO, 1996). *Triphyllochaeta vanelli* can be considered as the main parasite of the plumage of this bird species. Since no macroscopic feather damages were noticed and the intensity of infestation is considerably low (with less than 10 % of feathers bearing between 50 and 154 mites; see figure 1), feather mites can, according to SCHÖNE and SCHMÄSCHKE (2015), be regarded as commensals rather than parasites.

In the present study we evaluated a considerably high number of specimens of *T. vanelli*, showing a certain range regarding measurements of specific morphologic structures. For example MIRONOV *et al.* (2002) describe the length of the terminal cleft in males between 65-72  $\mu\text{m}$ . The parasites of the present study are consistent with this finding, showing a variation between 62-80  $\mu\text{m}$  (mean = 70  $\mu\text{m}$ , n = 334). Also the development of setae *h1* in females is subject to certain variability. MIRONOV *et al.* (2002) describe them as slightly enlarged. The present study shows a length between 12-29  $\mu\text{m}$  (mean = 22  $\mu\text{m}$ , n = 81) and therefore an appearance varying from pyramidal to spearhead-like. Still the main characteristics of *T. vanelli* according to MIRONOV *et al.* (2002) and GAUD and ATYEO (1996), separating it from very similar species, especially *T. paravanelli*, are fulfilled.

Further species of ectoparasites could not be detected in this study. This can probably be explained by the condition and age of the birds. Before examination lapwings were collected in the field, examined and euthanised, frozen and thawed. All of those steps decrease the chance of discovering a natural ectoparasite fauna that also includes chewing lice and ticks in the plumage. For research on feather mites this approach is nevertheless suitable since they show a very solid bond to their habitat. The study at hand made it possible to examine a larger amount of feathers and thus mites from *V. vanellus*, whereas earlier studies on the subject mostly rely on the examination of single feathers (SCHMÄSCHKE *et al.*, 2004; DABERT, 2000) or mite material collected in museums (MIRONOV *et al.*, 2002). The combination of these earlier findings with the present results allows for a validation and consolidation of knowledge regarding the feather mite fauna of the northern lapwing.

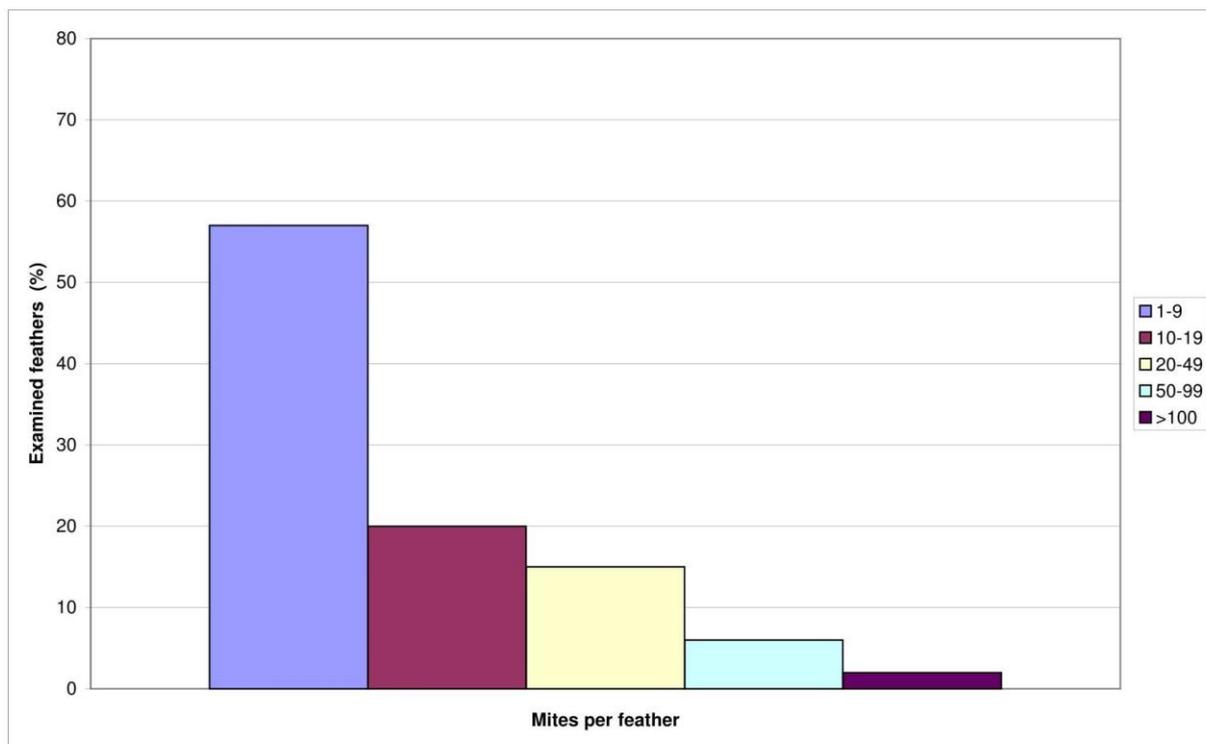


Fig.1: Intensity of feather mite infestation on feathers (n = 803) of lapwings (*Vanellus vanellus*) (Chart: Juliane Hintzen).

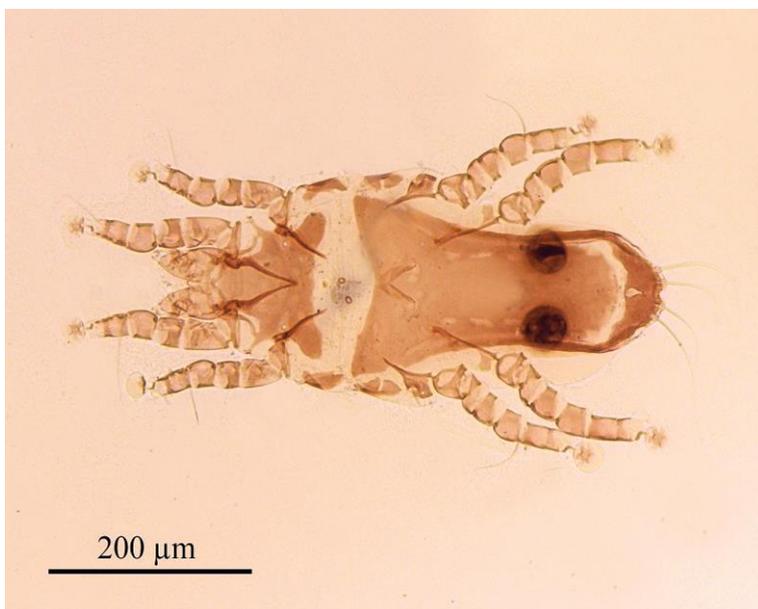


Fig.2: *Triphyllochaeta vanelli*, female (Image: Juliane Hintzen).

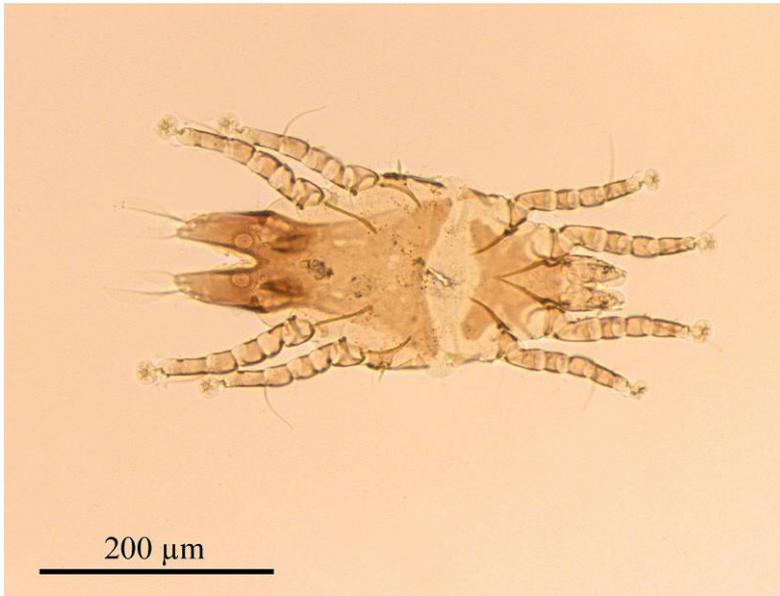


Fig. 3: *Triphyllochaeta vanelli*, male (Image: Juliane Hintzen).

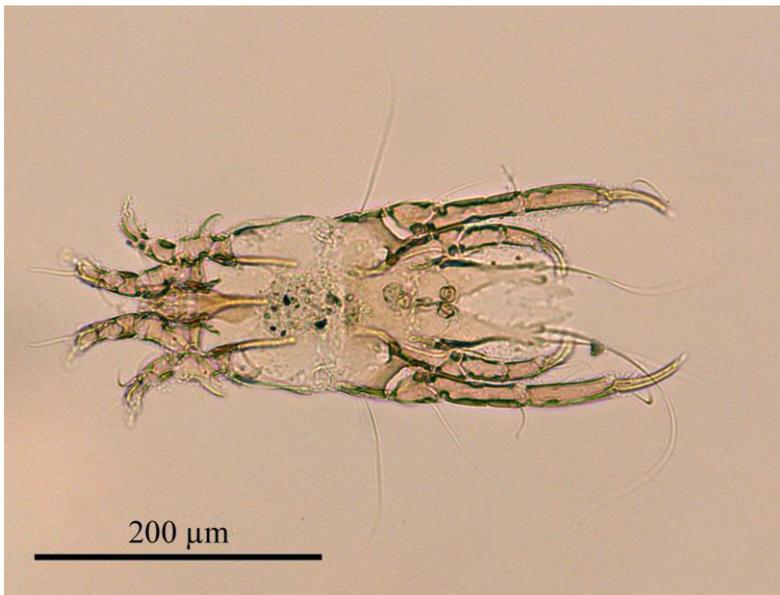


Fig. 4: *Leptosphyra centropoda*, male (Image: Juliane Hintzen).

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## SNOW LEOPARD CONSERVATION IN SPITI VALLEY, NORTHERN INDIA: WHAT A ZOO VET CAN LEARN DURING A VOLUNTARY SERVICE

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Zoo Basel is currently supporting ten nature conservation projects worldwide, all of them linked to species kept in its collection. Last year, nature conservation has been further strengthened. For every annual season ticket sold, a contribution of Swiss frank (CHF) 1.50 (approximately 1.4 Euro) goes to nature conservation projects, and there is a voluntary contribution of CHF 1.00 (approximately 0.94 Euro) added to all admission tickets. One of the supported projects is snow leopard conservation. The goal of the study was to get an on-site training in snow leopard (*Uncia uncia*) conservation work in Spiti Valley in the Indian Trans-Himalaya, and to share knowledge gained with this species ex-situ. The training was carried out in the Kibber Wildlife Sanctuary (33°N/78°E), where an indigenous agropastoralist community took the initiative to peacefully coexist with wildlife for nearly 20 years (MISHRA *et al.*, 2003). The conservation programme involves conflict management through a multi-pronged approach aimed at facilitating wild prey recovery by creating livestock-free areas, sharing and reimbursing economic losses due to livestock depredation by large carnivores through a community-run livestock insurance program, and improving the social carrying capacity for carnivores through sustained conservation education. Actions stretched from visits of snow leopard habitat to spot and count diverse wildlife species to quality controls of handicrafts produced by local women and corral reinforcements to protect livestock from snow leopards. Further involvement possibilities for zoo veterinarians such as assistance in veterinary management of domestic animals, feral dogs and wildlife have been investigated.

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## VETERINARY IMPORTANCE OF THE ELASMOBRANCH HUSBANDRY TRAINING IN THE BUDAPEST ZOO AND BOTANICAL GARDEN

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In the past decades training of lower vertebrates and invertebrates became an important routine all around the world in public aquariums. Budapest Zoo opened a new exhibit in 2012 with a 100m<sup>3</sup> stingray touch pool with four species of benthic stingrays (*Dasyatis pastinaca*, *D. chrysonota*, *Himantura uarnak*, *Neotrygon khulii*), common shovelnose rays (*Glaucostegus typus*), and brown banded bamboo sharks (*Chiloscyllium punctatum*). Because of its touch pool function and the overnutrition of the animals, we are faced with medical problems and stereotypical behaviours. Three common stingrays, four blue stingrays and one shark died. The cadavers were sent for necropsy to the Pathology Dept. of the University of Veterinary Medicine Budapest. The main necropsy and histopathological findings were fatty liver disease and egg retention. These are detectable with physical examination and sonography. The staff started to train the animals to prevent health issues. It was not only useful for the husbandry exercises, but also stopped certain stereotypical behaviours. Operant conditioning is a good method to work with elasmobranchs. In the beginning only primary reinforcers, such as food were used, but nowadays secondary reinforcers are also commonly applied. Target training is useful to move and separate animals, contact training sessions made the animals more calm and confident, less habitual/stereotyped. Currently the staff can make daily physical checks and shorter medical examinations like sonography without anaesthesia. In the future the authors plan to continue training the animals for blood draws, radiography, and explore new possibilities in their everyday life with creative training exercises.

## USE AND DURATION OF DESLORELIN ACETATE IN A *TESTUDO GRAECA* TO SOLVE A CHRONIC REPRODUCTIVE DISORDER

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### Summary

A 40-year-old female Mediterranean tortoise (*Taestudo graeca*) was presented for anorexia and ataxia each summer from 2009 to 2014. X-ray and ultrasound evaluation showed the presence of multiple eggs with poorly calcified shells each year. During this period, surgery or endoscopy or medical treatments with oxytocin and calcium injections were used to solve the dystocia and in the end, in 2014, it was decided to test treatment with Deslorelin. Despite Deslorelin being registered as contraceptive in adult intact male dogs and it has been used to control the reproduction in different species, there is no published data about its use in *Testudo graeca*. To evaluate the effect of the treatment, ultrasound examination of the coelomic cavity and ovarian follicle measurements were performed monthly for a period of two years, except during hibernation. Symptoms of reproductive disease were absent after treatment proving its effectiveness and 24 months after Deslorelin injection, the animal produced one egg.

To our knowledge, this was the first report describing the use of Deslorelin as treatment of a reproductive disorder in a reptile.

### Introduction

Since 1960, with the availability of orally active and increasingly more effective progestins in humans, efforts began to control reproduction in dogs and cats; for this reason, many types of progestins, oral and injectable, have been used for oestrus control in small animals and one approach in recent years has been the development of gonadotropin-releasing hormone (GnRH) analogues. GnRH is a hypothalamic hormone that has the same amino acid sequence in all mammals (Lucas, 2014). Modifications of the structure of GnRH provide two types of analogues: GnRH antagonists and GnRH agonists. Initially, the main disadvantage of the use of agonists was the need for frequent subcutaneous (SC) injections or SC mini-pumps over prolonged time periods to mimic the natural secretion of GnRH. In recent years, effective low-dose, slow-release implants containing potent agonists have been commercialised for use in veterinary medicine in the European Union (EU), such as Deslorelin implant (Suprelorin® Virbac, Carros, France).

Despite Deslorelin being registered as contraceptive in adult intact male dogs, during the years it has been used to control reproduction in a lot of other animals: bitches (LUCAS, 2014; VOLKMANN et al, 2006), tomcats and queens (LUCAS, 2014), male and female ferrets (SCHOEMARKER et al, 2008), birds (COWAN et al, 2014; PETRITZ et al, 2015), male bearded dragon (ROWLAND, 2011), rats (GROSSET et al, 2012), rabbits (GOERICKE-PESCH et al, 2015), wild carnivorous (cheetahs, lions) (BERTSCHINGER et al, 2001). In 2016 this implant was also used in reptiles to determine best practices for testosterone analysis in sea turtles (*Chelonia mydas* and *Caretta caretta*) (GRAHAM et al, 2016).

Even if the most common use of this hormone is to control reproduction, in the last few years it has been employed also to treat a wide range of reproductive conditions and diseases: in pet birds it has been applied to solve chronic egg laying, egg binding and excessive reproductive activities (MANS and PILNY, 2014); in guinea pigs it has been used to treat ovarian cysts (SCHUETZENHOFER *et al*, 2011).

The aim of the present study is to describe the use of Deslorelin to treat a reproductive disorder in a Mediterranean tortoise (*Testudo Graeca* - Linneus, 1758) and to see the duration of the implant. To our knowledge this is the first report describing the use of Deslorelin as treatment of a reproductive disorder in a reptile.

## Material and methods

Subject of this study was an approximately 40-year-old female *Testudo graeca*, weighting 2 kg. The subject was presented on June 2009 for anorexia and ataxia. X-ray and ultrasound evaluation showed the presence of multiple eggs with poorly calcified shells and blood analysis revealed a serious chronic infection. Medical treatments were ineffective and surgery was performed to remove eggs and multiple broken follicles (more than 100 mL of yellow foul-smelling liquid was aspirated). Two months later the animal completely recovered and came back to its yard.

In June 2010, the subject suffered once again from anorexia and diagnostic procedures revealed the presence of abundant liquid in coelomic cavity. Endoscopy was performed and the liquid aspirated; 1 hour after the subject was treated with Oxytocin (10 mg/kg IM. Sintocin®, Zoetis Italia, Roma, Italia) and Calcium (100 mg/kg IM. Calcium ph, Fatro, Ozzano Emilia, Italia) intramuscularly and some improperly calcified eggs of irregular size and morphology were expelled. The *Testudo graeca* was treated with Enrofloxacin (5 mg/kg/day IM. Baytril, Bayer, Italy) and after two weeks, symptoms completely disappeared.

In 2011, 2012 and 2013 the problem recurred and each time 10 mg/kg Oxytocin and 100 mg/kg Calcium were used as treatment and the tortoise improved.

In June 2014, when the tortoise was anorexic once again, informed consent was obtained from the owner and the authors decided to test Deslorelin as treatment to cure its chronic reproductive disorder.

After disinfection with chlorhexidine 0.5 % solution (Clorexan®, Cantel Medical, Pomezia, Italy), 4.7 mg Deslorelin implant (Suprelorin®, Virbac, Carros, France) was injected subcutaneously in the left hindlimb. Ultrasound examination of the coelomic cavity and ovarian follicle measurements were performed with monthly checks, except during hibernation, until the animal produced an egg (24 months after deslorelin injection). Examinations were carried out with a portable Esaote MyLab30 ultrasonograph (Esaote, Genova, Italy) using a 9.0 – 3.0 MHz micro-convex transducer probe. During the ultrasound scan, the subject was positioned on dorsal recumbency to facilitate the probe insertion, then the transducer was lubricated with ultrasound gel (GIMA, S.p.A., Gessate, Italy) and placed against the skin. The reproductive tract was identified from the prefemoral acoustic windows.

## Results and discussion

The ultrasound examination conducted before the Deslorelin implant showed absence of eggs, presence of three active, enlarged follicles ranging in size from 9 mm (smaller follicle diameter) to 21 mm (largest follicle diameter) and a corpus luteum of 38 mm in diameter. During the Deslorelin trial ultrasound exams revealed the absence of eggs and corpus luteum for the entire period of observation. The ultrasound scan, on day 21, detected evidence of a single vitellogenic follicle (27 mm

diameter). On day 85 the maximum diameter of the follicle was 23 mm. Enlarged follicles were observed on day 372 (n° 2; 15 - 11 mm), day 405 (n° 3; 16-14 mm) and day 442 (n° 3; 18-11 mm) suggesting a decrease in the ovarian activity compared to the condition the animal experienced during the previous years.

Twenty-four months after Deslorelin injection the animal produced one egg of normal shape and 1,7 x 0,9 mm diameter, though it was not physiologically layed; two months later Calcium and Oxytocin administration was needed again to solve the egg retention.

In conclusion these results demonstrate, for the first time, the effectiveness of Deslorelin treatment to control an endocrine reproductive disorder in a *Testudo graeca*.

The hormonal mechanisms underlying the follicular development in reptiles, as well as the coordination of deposition of the eggshell protein fibres by hormones and other mechanism have yet to be fully elucidated. Further studies are needed to completely identify the Deslorelin effects on the reproductive cycle of *Testudo graeca* to fully understand and control reproductive disorders.

## Acknowledgements

The authors thank Dr. Paolo Martelli and Dr. Douglas Mader for their advice.

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## VETERINARY ROLE IN THE REHABILITATION AND RELEASE OF WILD ANIMALS: THE MACAW EXPERIENCE

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In the 90's, work with wildlife in Costa Rica had limited veterinary involvement. It was hoarded by rescue centre owners, biologists and international consultants. The project Hospital de Especies Menores y Silvestres of the Universidad Nacional started generating changes in wildlife medicine. Within the areas engaged in was the recovery of wildlife populations, like the reintroduction of *Ara macao* in Piedras Blancas National Park, in Costa Rica. The macaws had disappeared because of the consequences of banana plantations, use of agrochemicals and hunting.

The project began with the formation of a group of macaws. They were clinically evaluated, tested with complete blood count, serum chemistry and screened for specific infectious agents such as polyomavirus, circovirus, newcastle disease virus, *Chlamydia* spp. and *Salmonella* spp. Once the birds were selected we moved them to a training cage and then further on to a pre-release cage to continue with natural feeding and for evaluation of behaviour and flight range. After verification of normal behaviour and health status we proceeded to the release. The first days the birds did not move far from the releasing place, but later they started flying longer distances and not returning frequently. Also, bird nests were placed near the release locations and three years later chicks were born in these nests. The population of today is continually growing and holds more than 200 individuals.

The macaw reintroduction project has strengthened the population of this species. This experience has been a role model to other reintroduction and rehabilitation projects of other species and is promoting interdisciplinary work to help wildlife.

## PARASITES OF FREE LIVING PASSERINES IN THE SOUTHERN REGIONS OF COSTA RICA

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### Summary

The object of this work was to identify the parasites of wild birds from the order Passeriformes in Gamba and San Vito regions of Costa Rica. In total 453 passerines have been captured with mist nets in the early morning, however only sedentary individuals (n = 219) were sampled. In order to characterize the parasite diversity and prevalence, faecal and blood samples were collected. After analyzing the blood smears, *Haemoproteus* was the most common blood parasite found. Endoparasite prevalence and diversity in faeces was low. All the birds evaluated show a good health condition. It is important to keep investigating the parasites ecology in these bird species, especially taking into account the importance of the region as wintering ground and migratory stopover sites.

### Introduction

According to BARKER *et al.* (2004), there are about 5,700 birds belonging to the order Passeriformes, which include most of the small birds, with sizes between a hummingbird (5.7-20 cm) and ravens (76 cm) (HARRISON and LIGHTFOOD, 2006, STILES and SKUTCH, 2007). Studying the parasites of different birds species is not only an important research topic of evolutionary ecology, but also for human and veterinary medicine as birds are known reservoir and vectors for different zoonotic agents. Costa Rica is an important biodiversity hotspot with more than 900 avian species (GARRIGES *et al.*, 2016), which potentially hosts abundant and diverse parasite communities. Despite the known importance of the southern regions of Costa Rica as wintering grounds or migratory stopover sites for several bird species, little information is available about the parasite diversity.

### Material and methods

The field work was from October to December in 2002, in November 2003, and in February and June of 2004. During the early morning hours, passerines were captured (n = 453) using mist nets (figure 1) however only sedentary individuals (n = 219) were sampled. Every bird was placed in a cloth bag and received a mash banana in order to avoid a hypoglycemic condition caused by the lack of alimentation. Birds were banded with an aluminum ring, blood and faecal samples were collected, after which all were released back at the site of capture. Blood samples have been collected from the jugular vein using a bird insulin syringe. For hemoparasites identification a blood smear was prepared, fixed with phosphate buffer (pH 6.8, AstralDiagnostics ®), dried, later stained with Giemsa in the Parasitology Lab of the Escuela de Medicina Veterinaria de la Universidad Nacional and analyzed under a microscope (40-100X). Faeces collected from each bird were deposited in Eppendorf tubes

containing saline solution. Samples were examined directly in saline solution and by floatation (modified Sheather with sugar solution) as described by Hernandez (2007).



Fig. 1: Capture and manipulation of sedentary birds in southern regions of Costa Rica.

## Results and discussion

Overall, 7.3 % (16/219) of the sedentary birds sampled were positive for hemoparasites (both *Haemoproteus* and microfilariae) (figure 2), while 5.0 % (11/219) had eggs or oocytes of endoparasites in their faeces (figure 3). While the prevalence and diversity of endoparasites was low in the different bird families and species (table 1), green honeycreeper (*Chlorophanes spiza*) showed a high prevalence in hemoparasites (table 1) although this could be attributed to the low number of individuals examined. Only two cases of co-infection were detected, both in Passerini's tanagers (*Ramphocelus passerinii*): one with *Capillaria* spp. and ascaridium and another one with *Haemoproteus* spp. and microfilariae.

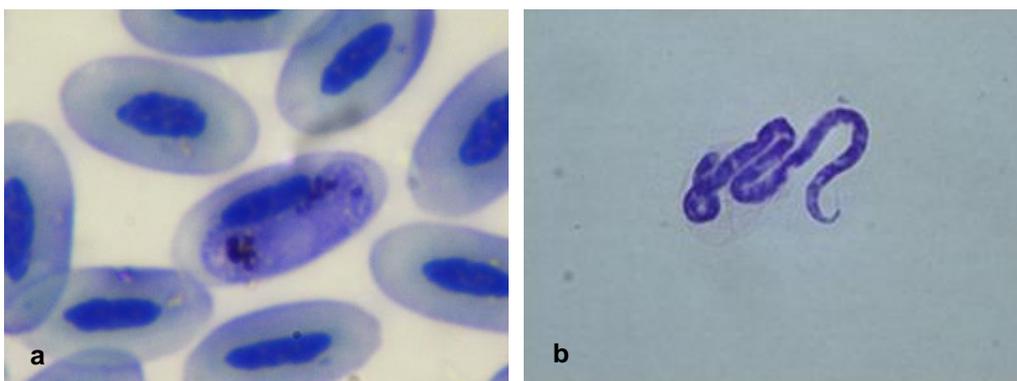


Fig. 2: *Haemoproteus* spp. (a) and microfilaria (b) detected in blood smears from sedentary passerines from southern Costa Rica. (100X).

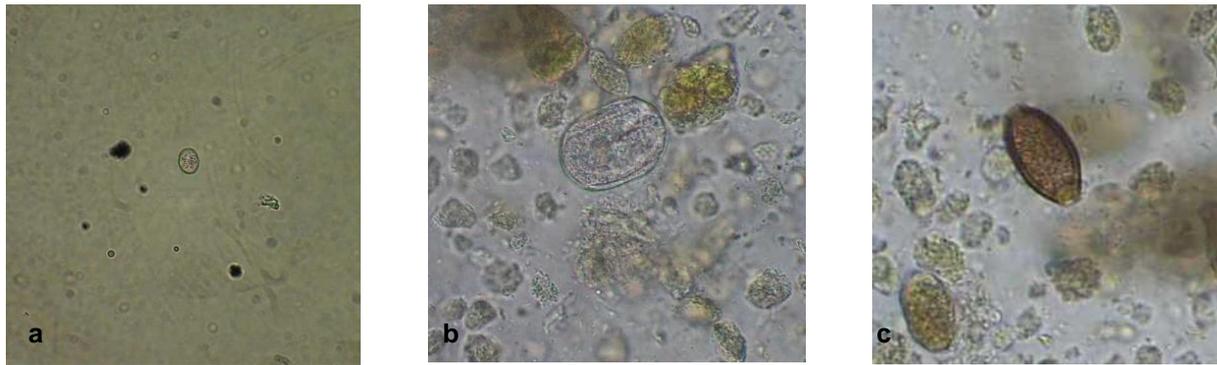


Fig. 3: Endoparasites in sedentary passerines from southern Costa Rica. (10X): coccidian oocyst (a), *Acuaria* spp. egg. (b) and *Capillaria* spp. egg (c).

Tab. 1: Hemoparasites and endoparasites of sedentary passerines sampled in the southern regions of Costa Rica

\* -  $n_{total}$  – number of birds sampled

-  $n_{+}$  - number of positive birds for the given parasite group

Family	Species	Hemoparasites		Endoparasites	
		% infection ( $n_{+}/n_{total}$ )*	Type of parasite ( $n_{+}$ )	% infection ( $n_{+}/n_{total}$ )*	Type of parasite ( $n_{+}$ )
Emberizidae	Black-striped sparrow ( <i>Arremonops conirostris</i> )	0 (0/13)	-	7,69 (1/13)	Coccidian
Emberizidae	Seedeaters ( <i>Sporophila</i> spp.)	3,33 (3/90)	<i>Haemoproteus</i> spp.	3,33 (3/90)	<i>Acuaria</i> spp. (2), coccidian (1)
Hirundinidae	Southern rough-winged swallow ( <i>Stelgidopteryx ruficollis</i> )	0 (0/14)	-	7,14 (1/14)	Coccidian
Thraupidae	Passerini's tanager ( <i>Ramphocelus passerinii</i> )	14,81 (8/54)	<i>Haemoproteus</i> spp. (8), microfilariae (1)	5,56 (3/54)	<i>Capillaria</i> spp. (4), ascaridium (1)
Thraupidae	Green honeycreeper ( <i>Chlorophanes spiza</i> )	50 (1/2)	<i>Haemoproteus</i> spp.	0 (0/2)	-
Thraupidae	<i>Thraupis</i> spp.	2 (1/20)	microfilariae	10 (2/20)	Ascaridium (1), <i>Capillaria</i> spp. (1)
Thraupidae	<i>Euphonia</i> spp.	3,33 (3/9)	<i>Haemoproteus</i> spp.	0 (0/9)	-
Turdidae	Clay-colored thrush ( <i>Turdus grayi</i> )	0 (0/17)	-	5,88 (1/17)	<i>Capillaria</i> spp.

Hemoparasites like microfilariae and *Haemoproteus* spp. can be detected frequently in different free-living bird species (RODRÍGUEZ-BARAHONA *et al.*, 2010; VALKIŪNAS *et al.*, 2004). In accordance to previous studies from northwestern Costa Rica (YOUNG *et al.*, 1993; VALKIŪNAS *et al.*, 2004), the most common hemoparasite observed in our study was also *Haemoproteus* spp. In sedentary birds from the order Passeriformes *Haemoproteus* spp. have been observed (RODRÍGUEZ-BARAHONA *et al.*, 2010) in the same region. *Plasmodium* spp. and *Leukocytozoon* spp. are other hemoparasites found in Northwestern Costa Rica (YOUNG *et al.*, 1993; VALKIUNAS *et al.*, 2004) but not in our study. Blood parasites may lead to bird's death by increasing the chances of predation and by decreasing survival during migration (SEBAIO *et al.*, 2012).

Previous studies on endoparasites of different wild birds reported a wide variety of nematode (*Ascaridia* spp., *Capillaria* spp., *Heterakis* spp., *Acuaria* spp., *Trichostrongylus* spp.), trematode and cestode eggs and protozoan oocysts (SLOSS *et al.*, 1994; RUPLEY, 1997; MCJUNKIN, 2003; KRONE, 2004). In the passerine species examined in this study, low endoparasite prevalence and diversity was found which might be related to the ecology of these bird species. Moreover it is important to mention, that studies on endoparasites of free-living passerines is relatively low.

Despite the detection of both parasite groups, no clinical effects were observed in the captured animals, all birds were in good condition.

Continuous monitoring of the avian parasites in these regions is warranted, in order to understand epidemiological connectivity between South and North America. Moreover, future studies should target both sedentary and migratory species sampled in different seasons and maybe expand the diversity of pathogens screened with bacteria and viruses due to the known epidemiological role of the wild birds.

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**SUDDEN DEATH OF TWO ADULT MALE CAPTIVE MASAI GIRAFFES (*GIRAFFA CAMELOPARDALIS TIPPELSKIRCHI*) WITH SEVERE RUMINAL BLOAT ASSOCIATED WITH SEROUS FAT ATROPHY AT A PRIVATE WILDLIFE RESCUE FACILITY IN EGYPT**

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Two adult, apparently healthy male Masai giraffes (*Giraffa camelopardalis tippelskirchi*) kept in captivity at a private wildlife rescue facility in Egypt, were found dead with no history of illness. Both deaths occurred in winter. The dead giraffes had severe ruminal frothy bloat which was suspected to be the immediate cause of death. There was also serous atrophy of multiple fat deposits and acute exudative interstitial pneumonia. All giraffes were fed with concentrates and dry alfalfa hay as the primary forage. Serous fat atrophy was probably due to inadequate energy intake and increased energy demands (respiratory disease and winter temperatures). Fibre intake in giraffe should be encouraged by restricting concentrated pellets, and palatable roughage and browse should be provided.

## DEVELOPMENT OF A DIAGNOSTIC PANEL OF REAL-TIME MULTIPLEX POLYMERASE CHAIN REACTION ASSAYS FOR SIMULTANEOUS DETECTION OF NEUROLOGIC PATHOGENS IN WILD CANID

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Rapid and reliable detection of the causative pathogens of Meningoencephalitis (ME) is essential for implementation of timely and effective therapeutic strategies. A panel of 3 multiplex real-time polymerase chain reaction (qPCR) assays was developed to detect the following major neurological pathogens in wild canids: bacteria, canine distemper virus (CDV), *Borrelia* spp., *Bartonella* spp., *Ehrlichia canis*, *Cryptococcus neoformans/gatti*, *Toxoplasma gondii*, *Neospora caninum*, and *Blastomyces dermatitidis*. The panel combined 1 reverse transcriptase-qPCR (RT-qPCR) and 2 qPCR. Each RT-qPCR and qPCR included three primer/probe sets for the target pathogens, one primer/probe set as an internal control (IC), and a reference dye. To minimize interference among the reporting dyes, each qPCR assay used the FAM, HEX, and Cy5 dyes to detect the agents: Cy3 for the IC and ROX for the reference dye. The analytical sensitivity of the panel was estimated using serially diluted recombinant vectors with known copy numbers per  $\mu$ l: 6.6 for the bacteria, 3.8 for CDV, 3.8 for *Borrelia* spp., 37 for *Bartonella* spp., 370 for *E. canis*, 3.7 for *C. neoformans/gatti*, 37 for *T. gondii*, 38 for *N. caninum*, and 66 for *B. dermatitidis* suggesting high analytic sensitivity. Standard curves showed correlation coefficients ( $R^2$ ) ranging from 0.984 to 0.999 and slopes of 3.27–3.57. No false positives due to cross-talk between dye signals or non-specific products were observed. Because treatment strategies for different histopathologic subtypes of ME are not specific, initial screening for infectious agents using the developed panel followed by CT or MRI would be useful for selecting an appropriate treatment in a timely manner. This subject is supported by Korea Ministry of Environment (MOE) as “Public Technology Program based on Environmental Policy (No. 2016000210002).

## CASE REPORT: LYMPHOMA IN LESSER HEDGEHOG TENREC (*ECHINOPS TELFAIRI*)

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Little information is known about neoplasia in Madagascar hedgehog tenrecs (*Echinops sp.*). This case report presents lymphoma in eight years old castrated male of Lesser Madagascar hedgehog tenrec (*Echinops telfairi*). The patient presented with a soft subcutaneous oval chestnut sized mass on the left side of the neck in the submandibular area. This animal was in good condition and clinically healthy. A fine needle aspiration biopsy was performed and cytology showed suspicion of neoplasia. Haematology and blood biochemistry parameters were within reference values. The mass was surgically excised and a sample was submitted for histology which confirmed the diagnosis of malignant lymphoma. Recovery was fast and healing of the surgical wound without complications. Two years later a tumour of similar size and texture appeared on the right side of the neck. After successful complete surgical excision, the mass was submitted for histopathology that resulted in the diagnosis of lymphoma most likely located in the thyroid gland. After immunohistochemical staining with primary antibodies against CD3 and CD79 $\alpha$  the majority of lymphoid cells showed immunoreactivity for CD3 and therefore lymphoma was classified as T-cell lymphoma. Recovery after surgery was uneventful, the surgical wound healed without complications and the animal showed no clinical signs for four years following second surgery. At this time the patient presented with sudden onset of apathy and in appetite. A walnut sized mass was palpable in the abdominal cavity. A renal tumour with multiple metastases was revealed during probatory laparotomy and the patient was euthanised. Histology samples obtained during the necropsy confirmed the diagnosis of adenocarcinoma with multiple metastases within the abdominal cavity. No pathological changes found during the necropsy indicated connection with the malignant masses removed during the two previous surgeries.

## **SURGICAL ATTEMPT IN A CASE OF A BROWN BEAR (*URSUS ARCTOS*) THORACIC DISC PROTRUSION**

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A 150 kg 40-year-old male Brown bear (*Ursus arctos*) was presented because of pelvic limbs weakness. The bear received prednisone 0.5 mg/kg (Deltacortene®, Bruno Farmaceutici, Rome, Italy) once a day daily for two weeks. The animal showed improvement but was found unable to walk when steroids were tapered down.

On neurological examination the bear showed normal mentation and paraplegia with normal deep perception. A thoracolumbar spinal cord lesion was suspected.

The bear was sedated with detomidine 0.02 mg/kg (Domosedan®, Janssen-Cilag, Cologno Monzese, Italy), tiletamine- zolazepam 2 mg/kg (Zoletil 50/50®, Virbac, Milan, Italy) IM; propofol 2 mg/kg (Propofol®, Merial, Milano, Italy) was administered IV to intubate him and maintained with 1.5 % Isoflurane (Isoflo®, Esteve, Bologna, Italy).

Serum biochemical and haematological analyses were within the normal values. Cerebrospinal fluid was collected by cisternal puncture, immediately prior to myelography and was considered normal compared with the reference value of the other mammalian species.

Myelo-CT was performed using 0.2 ml/kg of iohexol (Omnipaque®, Ge Healthcare, Milan, Italy) injected into the subarachnoid space via cisternal puncture and revealed a severe ventral spinal cord compression at T10-T11 intervertebral space consistent with a disc protrusion.

Immediately a decompressive surgery was performed. A left sided hemilaminectomy was done, the herniated disk was removed and disk fenestration was executed. The bear was kept in a small cage for a week and treated with enrofloxacin 5 mg/kg (Baytril®, Milan, Italy) and carprofen 2 mg/kg (Rimadyl®, Pfizer, Rome, Italy) for 10 days SID per OS. Two weeks after surgery minimal voluntary hindlimb movement were noticed. Because of the difficulty of handling and lack of recovery, the bear was euthanised six weeks after the surgery.

## AVIAN POXVIRUS INFECTION IN HUMBOLDT PENGUINS (*SPHENISCUS HUMBOLDTI*) AT THE ZOO AM MEER BREMERHAVEN, GERMANY

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### Summary

In a group of six three-month-old Humboldt Penguins (*Spheniscus humboldti*) at the “Zoo am Meer” Bremerhaven an avipoxvirus infection was diagnosed. After a short period of reddening of the eyelids all birds developed typical low- to high-degree nodular, verrucous and crusted skin lesions of the eyelids and of the featherless skin around the beak without reduced food intake or disturbed general condition. The diagnosis was based on histopathology, virus growth on the chorioallantoic membrane (CAM) of embryonated chicken eggs and the use of virus specific polymerase chain reaction (PCR). All diseased Penguins completely recovered in a period of two to three months.

### Introduction

Avian poxvirus has been reported almost worldwide in more than 270 bird species (BOLTE *et al.*, 1999; BOURNE *et al.*, 2012). Generally, it is assumed that the host range of a poxvirus is limited, but some of these viruses seem to have a broader host range (BOURNE *et al.*, 2012). The poxvirus may be transmitted indirectly by mechanical vectors such as insect bites or directly by contact of affected skin, mucosal membranes or infected parenchymatous tissue to injured skin of a potential host (KANE *et al.*, 2012). Avian poxvirus infections with pox lesions have been described in different penguin species in the wild, including the Gentoo Penguin (*Pygoscelis papua*) on the Falkland Islands (MUNRO, 2007), the Magellanic Penguin (*Spheniscus magellanicus*) in Argentina (KANE *et al.*, 2012) and Brasilia (NIEMEYER *et al.*, 2013) and the African Penguin (*Spheniscus demerus*; STANNARD *et al.*, 1998) as well as in captivity, in the Humboldt Penguin (*Spheniscus humboldti*) in North America and Europe (LANDOWSKA-PLASEWSKA and PLAZEWSKI, 1968; BOLTE *et al.*, 1999; GYURANECZ *et al.*, 2013).

### Case report

An isolated group of six three-month-old Humboldt Penguins (*Spheniscus humboldti*; one male, four females) at the “Zoo am Meer” Bremerhaven in Germany showed a reddening of the eyelids for about one week, developing after this time individually different low- to high-degree nodular, verrucous and crusted skin lesions of the eyelids and the featherless skin around the beak. The eyes, other parts of

the skin as well as the oral mucosa were not affected. The penguins were raised by their parents eight to nine weeks and then isolated in a small group behind the scenes as a routine procedure at the "Zoo am Meer". The food intake, development and general condition of all birds were not disturbed. Adult penguins or other bird species at the zoo were not affected.

The penguins were treated with systemic antibiotics (Enrofloxacin 15 mg/kg BID, 14 days; Baytril<sup>®</sup>, Bayer Vital GmbH, Leverkusen, Germany) and locally with an antibiotic eye ointment (Ofloxacin, BID, 14 days; Floxal<sup>®</sup>, Dr. Gerhard Mann chem.-pharm. Fabrik, Berlin, Germany). Until the skin lesions had healed a nourishing ointment (Bepanthen<sup>®</sup> eye and nose ointment BID; Bayer Vital GmbH, Leverkusen, Germany) was applied.

Complete healing of the skin lesions without scar formation took about 2 to 3 months. Complications were seen as fluorescein negative whitish irritation of the cornea, which healed up completely within the period of two to three months.

Conjunctival swabs and crusted skin material were taken from two penguins for microbiological examination. Pooled conjunctival, choanal and cloacal swabs of each bird were tested for *Chlamydia psittaci* by polymerase chain reaction (PCR, Vet Med Labor GmbH). Choanal and cloacal swabs as well as crusted skin lesions were tested for a herpesvirus infection by PCR. Biopsies (3 x 3 mm) of skin lesions of the eyelids and the beak of two penguins were taken under maskanaesthesia with Isoflurane (5 % induction, 3 % maintenance; CP Pharma GmbH, Burgdorf, Germany). The skin samples were fixed in 10 % neutral-buffered formalin for routine histopathological examination with hematoxylin and eosin (H&E) staining. Macroscopically altered skin material was inoculated in ten day-old embryonated chicken eggs for virus propagation and identification (WOODRUFF and GOODPASTURE, 1931). Macroscopically changed chorioallantoic membrane (CAM), harvest at seven days post inoculation, was examined by histopathology and PCR to detect pox virus DNA (GYURANECZ *et al.*, 2013).

### Results of the further laboratory investigations

The microbiological examination of conjunctival and crusted skin material revealed no pathogenic bacterial and fungal growths. *Chlamydia psittaci* as well as a herpesvirus infection were not detected by PCR. The histological examination displayed epithelial hyperplasia with hydropic degeneration and cytoplasmic eosinophilic inclusion bodies. Additionally a severe ulcerative to necrotizing inflammation with heterophilic infiltration was found. Macroscopically, the CAM of embryonated chicken eggs inoculated with material of skin lesions showed clouding and thickening and displayed histologically epithelial cell hypertrophy associated with cytoplasmic eosinophilic inclusion bodies and central lightening. Avipoxvirus specific DNA was detected by PCR and the nucleotide sequence of the amplicon was determined. The nucleotide sequence of a fragment of the pox DNA polymerase gene demonstrated a virus strain of the cluster 1 of clade B1 according to the classification of GYURANECZ *et al.* (2013).

### Discussion

The clinical signs and morphological findings in our cases are also termed dry pox and are similar to those described in the literature for poxvirus infections in penguins and other birds (BOURNE *et al.*, 2012; KANE *et al.*, 2012). However, wet pox characterised by diphtheritic lesions in the respiratory tract and in the oesophagus have been also reported in penguins, for example in Magellanic Penguins in Brazilian rehabilitation centres (NIEMEYER *et al.*, 2013), comparable to the respiratory form of canaries (JOHNSON and CASTRO, 1986). In our cases, there was no clinical evidence of respiratory disorder or swallowing problems. The clinical signs of poxvirus infections in birds mainly depend on the bird

species susceptibility and the virus virulence for the host as well as secondary bacterial and fungal infections (BOURNE *et al.*, 2012). Therefore, therapy is based on antibacterial and antifungal treatment and maintenance of the skin. The pathogenicity of the avipoxvirus in the reported cases seems to be reduced for Humboldt Penguins based on the observation that only young birds were diseased, skin lesions which healed well were found and the general conditions of the birds were not disturbed. According to the nucleotide sequence of a fragment of the DNA polymerase gene, the described avipoxvirus belongs to a group of viruses that were mainly isolated from a wide range of passerine species worldwide as well as in the Humboldt Penguin (USA, Spain) or the Magellanic Penguin (Brazilian) in captivity or wildlife rescue centres (NIEMEYER *et al.*, 2013; GYURANECZ *et al.*, 2013). Little is known about the host range of avipoxviruses. A primary European wild bird host as a virus source following interspecies transmission in the reported cases could be assumed. Avipoxviruses can be transmitted by insects, for example various species of mosquitoes. A mosquito plague was observed at the “Zoo am Meer” at the onset of clinical disease. Furthermore, possible transmission among the birds in the group may happen during the handfeeding process and the close contact of the juvenile penguins (BOURNE *et al.*, 2012).

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## ATRIAL SEPTAL DEFECT AND AORTIC VALVE STENOSIS IN A JUVENILE CHIMPANZEE (*PAN TROGLODYTES*)

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Although cardiovascular disease has attracted much interest in aged great apes, there is little information about cardiovascular problems in young animals. Here we describe a case of atrial septal defect and aortic valve stenosis in a 5 year old common chimpanzee (*Pan troglodytes*). Clinical history was uneventful up to a week prior to its death, when it showed signs compatible with respiratory virus infection (moderate respiratory and febrile signs) which also affected other group members. Although initial response to symptomatic treatment was observed, the animal was found dead 6 days after the onset of symptoms, and within 24 hours after a mild worsening of condition was observed. Gross findings included uncollapsed lungs, mild pleuropneumonia, and severe passive hepatic congestion. The heart presented bilateral hypertrophy, and complete atrial septal defect. The major histopathological findings were severe interstitial bronchopneumonia with 10 µm basophilic intracytoplasmic inclusions in pneumocytes and acute segmental fibrinonecrotising colitis with intralesional *Ballantidium* sp. Bacterial culture yielded *Stenotrophomas* sp growth in bronchial lymph nodes. No primary cardiomyopathy was detected; and Masson's trichrome stain was negative for myocardial fibrosis. Three-dimensional reconstruction of post-mortem computed tomography scan images of the heart, allowed demonstration of an atrial septal defect. Aortic valve stenosis with increased thickness of the myocardium of the left ventricle was also diagnosed. These findings are consistent with cardiopulmonary arrest secondary to complicated viral bronchopneumonia due to pre-existing severe cardiovascular compromise.

## DETECTION OF CHLAMYDIACEAE IN DIFFERENT SPECIES OF CHELONIANS

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Chlamydial species are increasingly recognised as infective and potentially pathogenic organisms in reptiles. Different types of symptoms were described in reptiles with *Chlamydia* sp. infections, usually with inflammatory lesions mostly in the spleen, heart, lungs and liver. Chlamydia can be isolated from clinically asymptomatic reptiles as well. To check for the presence of Chlamydia, 51 choanal and cloacal swabs were taken from different free-living and captive chelonians, 30 red eared sliders (*Trachemys scripta elegans*), 6 marginated tortoises (*Testudo marginata*) and 15 loggerhead sea turtles (*Caretta caretta*) during the routine health check up. The DNA was extracted by using QIAmp DNA Mini Kit (QIAGEN, Germany) and examined by *Chlamydiaceae*-specific Real-time PCR as described by EHRICHT *et al.* (2006). Positive samples were further examined by species specific Real-time PCR. Partial 16S rRNA genes were further sequenced. In total, 26 samples were found positive by *Chlamydiaceae*-specific Real-time PCR: 17 from red eared sliders, six from loggerhead sea turtles and three from marginated tortoises. Out of them, two samples from red eared slider were found positive for *C. psittaci* by species specific RT-PCR. Partial 16S rRNA gene sequencing revealed the similarity of one sample to the family *Waddliaceae* (96 % identity) and one sample to the family *Simkaniaceae* (93 % identity). Both of these samples were from loggerhead sea turtles. The role of chlamydial species in reptiles is still unclear, so that further investigation of uncharacterised Chlamydia and their impact to both reptile and human health is needed.

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## EFFECT OF A NEW GUM FEEDER OF CAPTIVE MARMOSETS AND TAMARINS: A COMPARATIVE APPROACH

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*Callitrichidae* in the wild feed on gums and saps, which are rich in carbohydrates and minerals. Gum is essential part of the diet of marmosets, whilst for the other callitrichid species it is of a limited importance. Nevertheless to fulfil their nutritional needs in captivity, *Callitrichidae* should be provided with gum Arabic, preferably given in a natural way to elicit species-specific behaviours. The aims of this study were: 1) to assess the effects of a wooden gum feeder on the welfare of two tamarin species (*Saguinus oedipus*, *S. midas*) and two marmoset species (*Callithrix geoffroyi*, *Cebuella pygmaea*); 2) to compare the duration of gum Arabic feeding between tamarins and marmosets. The studied subjects were eight tamarins (four cotton-top and four red-handed tamarins) and five marmosets (two pygmy and three Geoffroy's marmosets) housed in one enclosure per species. A continuous focal animal sampling was used to collect duration of individual and social behaviours over two periods: in the first period, gum Arabic was in bowls, in the second period each enclosure was provided with wooden gum feeders. Observations were recorded for a total of 480 min per monkey per period. Data were analysed using non-parametric tests and significance level was set at  $p < 0.05$ . First, the wooden gum feeder promoted species-specific behaviours such as exploration and decreased self-directed behaviours suggesting an enrichment effect of feeder on tamarins' and marmosets' behaviours. Secondly, even though there was a trend for marmosets spending more time feeding on gum than tamarins, the difference was not statistically significant.

## CT-SCAN VIRTOPSY AND NECROPSY CONFIRMED A YOUNG FEMALE PERSIAN LEOPARD (*PANTHERA PARDUS SAXICOLOR*) KILLED BY AN ADULT FEMALE

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### Summary

A female leopard which was captured on December 6<sup>th</sup> of 2015 and was fitted with a satellite GPS collar was found dead and partially eaten on January 30<sup>th</sup> of 2016, in fifty meters distance of an adult Urial ram. The Ground was covered with snow and tracks of at least two leopards, including footprints of a large specimen. During initial assessment of the dead leopard, luxation and fracture of cervical vertebrae was detected. CT-scan virtopsy carried out at Tehran University small animal hospital, on the same day and confirmed the fracture of the cervical vertebrae C2, C3, C4 and C5 in various extent. The leopard weighed 35.7 kg. Extensive subcutaneous and muscular hemorrhage were found on the left lower lateral side of the neck facing C3, C4, and C5. A comminuted fracture of the lower left part of the body of cervical vertebra C3 cortex and core, with protruding sharp-edged splints, as well as medial non-displaced point fracture of the body cortex of C4 were confirmed by necropsy. The leopard died from a comminuted fracture of the cervical vertebra C3 with puncture of the spinal cord and possibly a fracture of C4 which was confirmed by CT-scan virtopsy. CT-scan virtopsy and necropsy as well as footprints at the death site combined camera trap photographs indicated that the animal was killed by a larger-size leopard.

### Introduction

Virtopsy, virtual necropsy, of animals provides not only spectacular insight into the anatomy of the animals but also aids in the evaluation of the veterinary issues and determination of the cause and manner of death without the need for any manual dissection.

Intraspecific hostility is a common feature in large felids which exhibits agonistic behaviour toward their conspecific which is often leads to injury or death (BALME and HUNTER, 2004; GALENTINE and SWIFT, 2007). It may be either intrasexual or intersexual and affects all cohorts. It is an important source of mortality among leopard populations globally (*Panthera pardus*) (BALME *et al.*, 2009) and may be associated with competition over a kill (STEYN and FUNSTON, 2006), cannibalism over territory (BALME

and HUNTER, 2004) and infanticide (BALME *et al.*, 2009; BALME and HUNTER, 2013). Infanticide is only committed by males and previous studies suggest it is the leading cause of cub mortality (BALME *et al.*, 2013; SWANEPOEL *et al.*, 2015).

Nevertheless, the occurrence of intraspecific aggression among adult leopards is poorly understood from Asian populations.

## Cases

This report forms part of ongoing research on leopard spatial ecology initiated in 2014 in Tandoureh National Park, north-eastern Iran (ca. 20 km from Turkmenistan border).

During this project, five adult leopards (four males and one female) have been cut with Aldrich foot snares (FRANK *et al.*, 2003) modified to soften the consequences of struggles, remotely monitored with VHF trap transmitters (Wildlife Materials, Inc., Illinois, USA). Anaesthesia was safely achieved using a combination of Ketamin 10 % (Alfasan, Nederland BV) 3 mg/kg and Medetomidine HCL 20 mg/ml (Kyron Laboratories (Pety) Ltd., Johannesburg, South Africa) 30 µg/kg administered IM in the same 1.5 ml dart by Daninject darting gun (Daninject, Denmark). The darting sites were biceps femoris except one which was triceps brachii. Capture-related injuries included minor cuts and abrasions to the feet. Lotek Iridium GPS collars (Lotek Wireless Inc., Ontario, Canada) were used, each supplemented with a drop off buckle with timer only option (working after 52 weeks since deployment) that automatically removes the collar, so that re-capture of animals is unnecessary. Collars used weighed 640 g; equivalent to less than 1 % of body mass for captured leopards.

The female leopard in question was caught on December 6, 2015 (17:15) and was estimated to be three years of age at a trap with shot boar hung as bait. The anaesthesia period took 44 minutes, followed by reversal using Atipamezole 5 mg/ml (Syva S.A., Spain). The capture was uneventful, and the animal recovered well from the anaesthesia. No broken teeth or claws and no leg injury were recorded during the procedure.

On 30 January 2016 (10:30), a mortality signal emitted from a rocky slope was obtained. At 17:00 on the same day, local rangers found the GPS-collared animal dead just 50 meters from an adult (> 5year-old) Urial ram (*Ovis orientalis vignei*), partially eaten. The ground was covered with snow and tracks of at least two leopards, including footprints of a large specimen were visible. One day later, a Bushnell Trophy camera trap (Kansas City, Missouri, USA) was deployed at the kill site. Two days later, the kill site was visited to collect the camera trap. The Urial kill was completely eaten by an adult leopard photographed by the camera trap.

The dead leopard was transported to the central office of the Dept. of Environment (DoE) in Tehran on 2 February 2016 for necropsy and further investigations. The external examination revealed an animal in good body condition with no major skin lesions. When manipulating the neck, a luxation and fracture of cervical vertebrae was detected.

## Results

X-ray computed tomography (CT scan) carried out at Tehran University small animal hospital, on the same day and confirmed that cervical vertebrae C2, C3, C4 and C5 were fractured to various extent (figure 1). A comminuted fracture (the bone was crushed with more than three fragments) of the lower left part of the body (cortex and core) of cervical vertebra C3, with protruding sharp-edged splints, as well as possible medial non-displaced point fracture of the body cortex of C4 were confirmed. The fracture of C3 was combined to a perforation of the spinal cord canal.

Gross necropsy examination procedure of the necropsy of the animal followed a standard procedure. The animal weighed 35.7 kg, extensive subcutaneous and muscular (sternohyoid and sternomastoideus muscles) hemorrhage on the left lower lateral side of the neck facing C3, C4, and C5 were found (figure 2).

The presence of associated left lateral and ventral distinct perforations of the neck muscles combined to extensive hemorrhage support the possibility of a predator bite (maxillary canines) at the origin of these multiple fractures. The double puncture was related to superficial and deep hemorrhage as well as to cervical vertebra fractures. The distance between puncture holes was 4.9 - 5.0 cm and corresponded to the rostrum breadth in adult female Persian leopards, ranging 4.32 to 5.49 cm (N = 16) (FARHADINIA *et al.*, 2014). No major external injuries were visible; eyes, nostrils, ears, anus orifices were all normal. The animal presented a small non-gravid uterus without any placental scar of a past pregnancy.



*Fig. 1: 3D CT scan revealed cervical vertebrae C2, C3, C4 and C5 had comminuted fractures with two holes at the ventral aspect. The distance between puncture holes was 4.9 - 5.0 cm (Images: Mohammad Molazem).*



*Fig. 2: Extensive subcutaneous and muscular (sternohyoid and sternomastoideus muscles) hemorrhage on the left lower lateral side of the neck facing C3, C4, and C5. (Photo by: Alireza Shahrhiri Panah)*

## Discussion

The comminuted nature of the fracture, which typically requires considerable force and energy, the distance between puncture holes in the neck muscles, likely due to upper canines, the presence of a freshly killed prey in the vicinity of the scene along with the presence of footprints belonging to a large leopard at the site of the kill supported that the female was killed by a larger leopard. The deployed camera trap filmed an adult leopard on site. Field investigations indicated that the adult non-collared leopard was on the kill when the collared individual approached her. Based on tracks left on the snow, the collared leopard was chased at least twice. We were not able to judge who killed the Urial ram, but their conflict over the kill was evident. Competition over food is the likely cause of the reported intraspecific killing in Tandoureh.

To the best of authors knowledge, this is the first documented report of intraspecific killing among free-ranging leopards in Asia. In Africa scientists (STEYN and FUNSTON, 2006) noted an adult male leopard killing and consuming an adult female in Botswana's Tuli Block. This instance appears to have been related to a kill which the female made. That was in savannah environments where preferred leopard prey and female leopard was abundant (STEYN and FUNSTON, 2009; FATTEBERT *et al.*, 2016). Our record is notable in light of the low density, elusive behaviour and observational difficulties of leopards in Iran where individual interactions rarely allow scientists to understand the cause of intraspecific mortality of leopards.

This young female Persian leopard died 61 days after being captured in Tandoureh National Park. The laps of time between the capture and the death (nearly nine weeks), the good body condition, the lack of visible lesions of the snared foot (left carpus joint), of teeth and claws, and the fair amount of fat deposits (supporting normal foraging) exclude the possibility that the capture and anaesthesia have been responsible for a weakening of the animal or have been directly responsible for the death (figure 3).



*Fig. 3: The fair amount of subcutaneous abdominal and kidney fat deposits (supporting normal foraging) exclude the possibility that the capture and anaesthesia have been responsible for the weakening of the animal or have been directly responsible of the death. (Photo by: Alireza Shahrhiri Panah)*

The capacity to hunt, the capacity to swallow bone fragments, the absence of cutaneous, subcutaneous or muscular lesions associated with the collar and a good body condition exclude the possibility that the collar could have been responsible for the weakening of the animal or has been

directly responsible for the death. The slight decrease (3.5 %) in body mass compared to the measurement taken during capture is compatible with a post-mortem body water loss during cool storage (seven to eight days between death and necropsy). The animal died per acutely from a comminuted fracture of the cervical vertebra C3 with a perforation of the spinal cord canal and possibly also a fracture of C4 confirmed by CT scan virtopsy.

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## CLINICAL CASES OF NECROTIZING FASCIITIS IN BENNETT WALLABIES (*MACROPUS RUGOGRISEUS*) BETWEEN 2005 AND 2015 AT ZOOPARC DE BEAUVAL, FRANCE

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Bennett wallabies (*Macropus rufogriseus*) are quite common macropods in zoological institutions. Considered as “Least concern” by IUCN, they typically have a good reproductive status, live as multiple males/multiple females in a group and are found in enclosures or children pet farms. Known to be sensitive to toxoplasmosis and oral necrobacillosis, issues with bite wounds can often be encountered during breeding season.

Necrotising fasciitis, also called flesh-eating disease, is an infection of the deep layers of cutaneous and subcutaneous tissues. The infection spreads along the fascia and rapidly progresses. The infection is usually rare and mainly due to group A *Streptococcus*, *Vibrio vulnificus*, *Clostridium perfringens*, *Bacteroides fragilis*, *Fusobacterium* sp or *Porphyromonas* sp. Treatment is based mainly on very broad surgical debridement of necrotic cutaneous and subcutaneous tissues to block spreading of the responsible organisms and associated high dose antibiotic administration. Treatment success depends on rapid intervention and without treatment, the outcome is always fatal.

Between 2005 and 2015, ZooParc de Beauval experienced eight cases of necrotising fasciitis within its Bennett wallaby population which will be summarised here with an emphasis on two successes. Despite the keepers’ goal to detect any signs at an early stage, the fur of the animals and the particularity of the infection of initial subcutaneous lesions hinder the latter and prevent early care of the infected animals. Surgical debridement impairs the recovering ability. Even if the infection itself is resolved, the number and frequency of anaesthetic events usually induce a stress, which may compromise the overall outcome.

## SATELLITE TRACKING OF REHABILITATED SEA TURTLES IN THE SOUTH CHINA SEA

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Sea turtles are globally endangered species and face anthropogenic threats of all kinds. The National Museum of Marine Biology and Aquarium (NM MBA) regularly receives stranded sea turtles in need of medical treatment from public reports in southern Taiwan. *Chelonia mydas* is the most common species encountered. These sea turtles are treated and rehabilitated at the NM MBA, and then released when considered physically fit. To understand the post-release movements and survivorship of these turtles, and to enrich knowledge of the foraging grounds of sea turtles in the South China Sea, we have developed since 2013 an on-going collaborative project to satellite track the rehabilitated sea turtles. A satellite transmitter was attached to the carapace of each suitable turtle with fiberglass resin protocols described by BALAZS *et al.* (1996). The weight of the transmitter package was less than 5 % of the body weight of the turtles to minimize potential impact to the turtles. Tracks were plotted using only the most representative and accurate daily location points by Maptool (SEATURTLE.ORG, Inc. <http://www.seaturtle.org/maptool/>). To date, ten sea turtles, including eight green turtles and two olive ridley turtles, were tagged with satellite transmitters and released. Nine of the trackings ended in their foraging grounds, except that one turtle was possibly stranded after release. The above trackings revealed that rehabilitated sea turtles survived well in the wild. This project also enriches our understanding on the finer-scale distribution of foraging grounds of green turtles and olive ridley turtles in Taiwan (in particular southern and eastern Taiwan), the South China Sea (i.e. the Philippines). We encourage consistent efforts be devoted in the research of wildlife rehabilitation and management.

## INTESTINAL OBSTRUCTION DUE TO COLIC FAECAL STASIS IN THREE CAPTIVE OKAPIS (*OKAPIA JOHNSTONI*)

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Three adult okapis (*Okapia johnstoni*) died from intestinal obstruction at ZooParc de Beauval between 2009 and 2017. Case 1 concerned a 19-year-old male which presented with dysorexia and constipation for two days. Transrectal examination was unremarkable. No general anaesthesia was performed in this case. The animal was euthanised after two days due to worsening health status. Case 2 was a 19-year-old female with history of dystocia and abortions. No faeces were passed despite four days of medical treatment. General anaesthesia was performed on day 3 for further examination. Transrectal examination revealed scant faeces surrounded by mucus in the rectum. This animal died the day after anaesthesia. Case 3 concerned a 21-year-old male which had been anaesthetised two days earlier for preshipment examination. This animal presented with dysorexia and constipation. At day 2 and 3, the animal was given oral sorbitol and paraffin oil on hay and received intramuscular injections of flunixin meglumine and metamizole, without any result. Sedation was performed after 4 days and intravenous fluids were provided. At day 6, slight aerial dilatation of the small intestines from undetermined origin was detected during exploratory laparotomy. The animal died the day after surgery. All necropsies revealed obstruction due to faecal impaction in the spiral colon, necrosis of the surrounding bowel, and moderate to severe dilatation of orad intestine. As in giraffe (DAVIS *et al.*, 2009), these cases highlight the difficulty of ante-mortem diagnosis and treatment, and poor prognosis of spiral colon obstruction in okapis.

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## ORTHOPEDIC FRACTURE REPAIR IN A DAMA GAZELLE (*NANGER DAMA*)

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### Summary

Wild bovids kept in captivity are prone to trauma which often results in complications causing debilitation, infection and mortality. Fractures relating to trauma are commonly encountered and metacarpal / metatarsal fractures are reported in 50 % of incidences in domestic ruminants. In zoological medicine the management of orthopedic conditions in captive wild bovids is under-reported in the veterinary literature. External Skeletal Fixation (ESF) is considered a useful technique in fracture repair in many species including small ruminants. The use of an ESF to repair distal limb fractures of ruminants is preferable when external coaptation is considered inappropriate. This paper describes the surgical repair of a closed, mid-diaphyseal, short oblique, comminuted metacarpal fracture in a male sub-adult Dama Gazelle (*Nanger dama*). The surgical implantation of an improvised type II ESF is described as are several complications encountered in recovery which resulted in development of a moderate acquired angular limb deformity.

### Introduction

Wild bovids kept in captivity are prone to trauma which often results in complications causing debilitation, infection and mortality. Fractures relating to trauma are commonly encountered and metacarpal / metatarsal fractures are reported in 50 % of incidences in domestic ruminants. In zoological medicine the management of orthopedic conditions in captive wild bovids is under-reported in the veterinary literature. (WOLFE, 2015; ANDERSON & JEAN, 2008)

This paper describes the surgical repair of a closed, mid-diaphyseal, short oblique, comminuted metacarpal fracture in a male sub-adult Dama gazelle (*Nanger dama*). The application of an improvised type II ESF (external skeletal fixation) is described as are several complications encountered in recovery which resulted in development of a moderate acquired angular limb deformity.

### Material & methods / Case report

In November 2015 the animal presented acutely with a mid-diaphyseal fracture of left metacarpal bone. The animal was immobilised by remote injection with a Dan-inject® CO<sub>2</sub> powered rifle with an anaesthetic combination of 8 mg Butorphanol (0.32 mg/kg, Kyron Laboratories, RSA), 10 mg Azaperone (0.4 mg/kg Kyron Laboratories, RSA), 2 mg Medetomidine (0.08 mg/kg, Kyron Laboratories, RSA). The animal was stabilised for radiographs. Intravenous fluids were administered during the procedure 1,500 ml at maintenance rates (6.35 ml/min). The animal was not intubated, O<sub>2</sub> at 2 l/min was delivered by nasal insufflation. Body weight was estimated at 25 kg. Antibiotic (375 mg amoxicillin, 15 mg/kg (Betamox L.A., Norbrook Laboratories Ltd., Northern Ireland)) and a non-steroidal anti-inflammatory (100 mg tolfenamic acid, 4 mg/kg (Tolfejec, Troy Laboratories, Australia)) were administered peri-operatively.

Radiographs confirmed a closed mid-diaphyseal short oblique comminuted fracture of the left metacarpal bone. Because the animal was a genetically valuable specimen the decision was made to immediately attempt a surgical fixation of the fracture. The animal was not considered for external coaptation by splint or casting as it would not tolerate a cast that would impede movement of the limb. The animal was prepared for surgery and anaesthesia was maintained by intermittent intravenous administration of boluses of ketamine (140 mg total, 1 – 2 mg/kg dose, Ketamil, Troy laboratories, Australia), butorphanol (16 mg total, 0.3 mg/kg, Kyron Laboratories, RSA) and medetomidine (2 mg total, 0.08 mg/kg, Kyron Laboratories, RSA). Despite the unavailability of ideal materials an ESF device was improvised using 4, 3.2 mm X 230 mm Steinmann intramedullary pins, 1.2 mm (16g) cerclage wire and veterinary thermoplastic (Imex Veterinary Inc., USA).

Lateral stab incisions were made on the skin proximal and distal to the bone fragments, the fascia was separated and the bone was drilled transversely using a commercial cordless drill disinfected with surgical spirit and wrapped in sterile vet wrap. The drill exit sites were identified by placement of 18g needles and skin was incised before final pin placement. The device was configured as a type II, bilateral uniplanar ESF device. 4 IM pins were placed transcortically across the metacarpal bone in the medial-lateral plane. The 4 pins, 2 pins proximally and 2 pins distally, were spaced approximately 2 cm proximally and distally from the fracture and 2cm from each other. The ends of the pins were bent to 90° and interlocked and secured with cerclage wire and orthopedic thermoplastic. Upon completion of the procedure post-operative radiographs were taken.

As the animal was moved for post-operative radiographs it was aroused spontaneously. It made several forceful blunt impacts of the fracture and ESF on the table, before being manually restrained and before an additional 30 mg ketamine bolus could be administered intravenously. Post-operative radiographs indicated misalignment of the fracture. A step was palpable on medial aspect across the fracture and mild/moderate valgus deviation was also visibly evident. The decision was made to recover the animal as the ESF was stable.

The extremely nervous disposition of the animal meant that any further intervention was kept to a minimum as it reacted negatively whenever approached or observed. The animal was allowed to recuperate in quiet isolation. Antibiotic and anti-inflammatory treatment was repeated 48 hours post operatively by remote injection. After one week it started to bear weight on the leg.

## Results & discussion

After eight weeks it was immobilised for further radiographs (using a combination of 1.5 mg etorphine (0.05 mg/kg, M99, Novartis, RSA) and 3 mg medetomidine 2 % (0.1 mg/kg Kyron Laboratories, RSA); reversed with 40 mg naltrexone (Kyron Laboratories, RSA) and 15 mg antipamazole, (Troy Laboratories, Australia)). Callus formation and bone remodeling was reported, however there was extensive periosteal proliferation around the fracture site with misalignment of fracture and moderate valgus deformity. Final ESF removal made at 14 weeks under anaesthesia using the same combination as above.

ESF is considered a useful technique in fracture repair in many species including small ruminants. Despite low body weight and readily availability of suitable orthopedic implants similar size to human or large breed dog, the adaptation of ESF as a means of fracture repair in small ruminant species has been slow (STAUDTE & GIBSON, 2003; ANDERSON & JEAN, 2008).

The use of an ESF to repair distal limb fractures of ruminants is preferable when external coaptation is considered inappropriate (i.e. when half leg or full leg casts would not be tolerated by the animal). ESF minimises soft tissue injury due to anatomy of distal limbs; provides excellent mobility and early return of function; limits invasive surgery at fracture site allowing local blood flow and osteogenesis; diverse

applications for treating various fracture types; and permits easy implant removal after clinical union achieved (JEAN & ANDERSON, 2014; PENTECOST, *et al*, 2016).

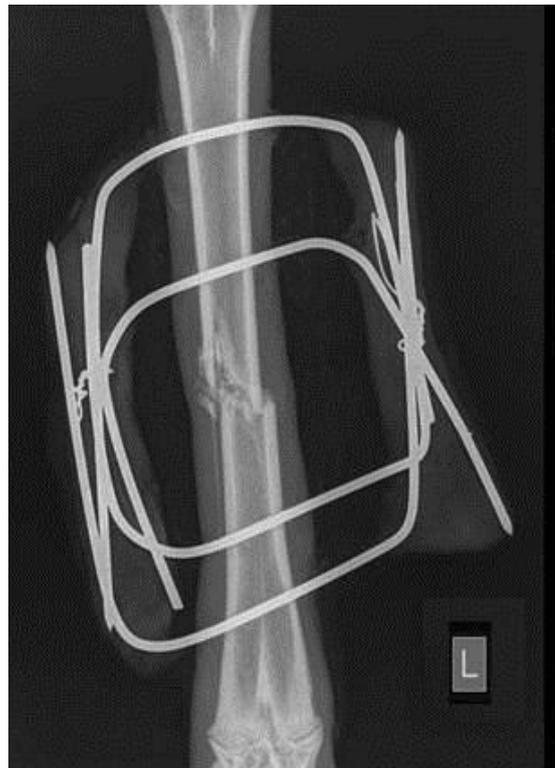
Disadvantages of ESF are suboptimal fracture reduction; absence of compression across the fracture; reduced biomechanical stability in comparison to internal fixation; and motion of the pins at the bone interface may cause pain or predispose to development of osteomyelitis (NEWMAN & ANDERSON, 2009; JEAN & ANDERSON, 2014).

Aside from the nervous disposition of the patient, other factors may have contributed to the development of acquired valgus deformity: the fixation method chosen did not allow for optimal compression and reduction of the fracture and potential inaccurate transcortical pin placement due to inexperience of the veterinary surgeon that may have resulted in unequal biomechanical forces acting across the ESF (JOHNSON & HULSE, 2002; STAUDTE & GIBSON, 2003).

A recent opportunistic radiograph revealed extensive cortical bone remodeling and periosteal reaction. The extensive periosteal reaction across fracture site was probably due to excessive osteogenesis and extensive bone remodeling in recovery or possibly a self-limited localised osteomyelitis (STAUDTE & GIBSON, 2003). The cortical bone has calcified sufficiently and is biomechanically robust. The procedure was considered a success despite the malunion and moderate valgus deformity. However the animal may be predisposed to develop arthritis in later years.



*Fig. 1: Dorso-ventral radiograph of left metacarpus. Note short oblique, comminuted mid-diaphyseal fracture (© Al Ain Zoo).*



*Fig. 2: Post-operative radiograph. DV orientation. Note fracture mal-alignment and configuration (© Al Ain Zoo).*



Fig. 3: Post Recovery radiograph. DV orientation. Note extensive periosteal reaction along left metacarpal bone and cortical bone remodeling. Radiograph includes right metacarpal bone for comparison(© Al Ain Zoo)

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## ROOT CANAL TREATMENTS AND CROWN LENGTHENING IN AN ADULT JAGUAR (*PANTHERA ONCA*)

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### Summary

Pathological dental conditions in carnivores are frequently encountered though under-reported medical conditions of zoo and free ranging wild animals. Teeth fractures, especially canine tooth fractures due to trauma, are most commonly encountered and often require specialised endodontic intervention. This paper describes gingivectomy of a peripheral odontogenic fibroma of the left maxillary gingivae; crown lengthening of the left mandibular canine; and pulpectomy, or non-surgical root canal treatment, of complicated crown fractures of the left maxillary canine tooth (204), left mandibular canine tooth (304) and left third maxillary incisor (203) in an adult jaguar (*Panthera onca*). Due to limited opportunity to make routine clinical assessments in zoo species, oral and dental examination and prophylaxis should form part of the examination procedure during any opportunistic intervention. In cases where further dental intervention is indicated, e.g. endodontic surgery, there are problems relating to availability of specialised materials, specialised equipment and, depending on clinical experience of the attending veterinarian, specialised medical or surgical assistance. These limitations are as relevant to zoological medicine today as when described by Fowler in 1986.

### Introduction

Pathological dental conditions in carnivores are frequently encountered, though under-reported, medical conditions of zoo and free ranging wildlife animals. Teeth fractures, especially canine tooth fractures due to trauma are most commonly encountered and often require specialised endodontic intervention. (FURTADO, *et al*, 2007; GLATT, *et al*, 2008)

Endodontics is the branch of dentistry that deals with the diagnosis and treatment of diseases of the pulp and their sequelae in the apical, periapical, radicular and periradicular tissues (WIGGS & LOBSPRICE, 1997). When pulpal tissue is compromised and its vitality threatened or destroyed, some form of endodontic treatment is necessary to preserve teeth that might otherwise be exfoliated or extracted (WIGGS & LOBSPRICE, 1997). Therapy may also serve to resolve dental and periodontal infections that can result in local and systemic complications (PAVLICA, *et al*, 2008).

This paper describes the root canal treatment (RCT) of left mandibular canine, left maxillary canine teeth fractures, 3<sup>rd</sup> left maxillary incisor fracture in an adult female jaguar (*Panthera onca*). A gingivectomy of a gingival fibroma obscuring the maxillary canine fracture, and a crown lengthening procedure of the mandibular canine fracture are also described.

### Materials & methods / Case report

In April 2013 an adult female jaguar was diagnosed with left maxillary canine and left mandibular canine complicated crown fractures with a cauliflower like hyperplastic gingival growth completely

obscuring the base of the left maxillary canine tooth, biopsy of the hyperplastic mass was performed. Endodontic surgical intervention was recommended.

In June 2015 the animal was anaesthetised for oral examination, radiography and endodontic root canal treatment by visiting specialist veterinarians. Anaesthesia was induced by single intramuscular injection by dart using a Dan-inject blow-pipe. An anaesthetic combination of ketamine (Ketamil, Troy laboratories, Australia, 100 mg/ml at 2 mg/kg), medetomidine (Kryon Pharmaceuticals South Africa 20 mg/ml at 0.03 mg/kg) and butorphanol (Kryon Pharmaceuticals South Africa, 50 mg/ml at 0.2 mg/kg) resulted in induction after 10 minutes. Intravenous fluids were administered during the procedure (1000 ml Ringers Lactate administered over 2 hour procedure at rate of 8.3 ml/min i.e. 3 drops/s). The animal was intubated and maintained on Isoflurane at 2 %, O<sub>2</sub> flow of 2 l/min using an open circuit circle system. Anaesthesia was monitored initially at 5 then 10 minute intervals by assessment of vital parameters (temperature, heart rate and respiration rate), anaesthetic depth (graded 1-5) and by pulse-oximetry (SpO<sub>2</sub>).

Following oral examination and dental chart assessment, a mass present buccally on the left maxillary canine gingiva, complicated crown fractures of the left maxillary canine tooth (204), left mandibular canine tooth (304) and left third maxillary incisor (203) were diagnosed. Pre-operative extra-oral rostral maxillary and mandibular radiographs (oblique and dorso-ventral views) indicated no inflammatory root resorption or periapical pathology was detected.

Complete periodontal prophylaxis was conducted by manual supra & sub-gingival scaling, including gingival sulcus lavage with 0.12 % chlorhexidine solution, and tooth polishing with commercial polishing agent.

A gingivectomy of the gingival mass was performed to expose the maxillary canine tooth fracture site, and the excised mass placed in formalin. Gingival sulcus depth was measured using a periodontal probe. Bleeding point estimation of the gingivae was made using a small gauge needle to guide the excision of the gingivae. Haemostasis was achieved by direct application of pressure at the incision site.

In order to increase the clinical crown length of 304, surgical crown lengthening was performed: a mucoperiosteal flap was created by stripping the gingiva from the bone with a periosteal elevator; an alveoloplasty of 304, using a tungsten round bur size 4 was performed; finally, the mucoperiosteal flap was apically repositioned and secured by 4/0 Monocryl sutures. (HOLSMETROM, 2013)

In the absence of radiographic lesions, a standard root canal therapy (pulpectomy) was performed for both canine and maxillary incisor fractures. All the teeth were prepared through a normograde approach. The diseased pulp was removed through the coronal fracture sites using various Hedstrom files. The prepared teeth were disinfected by irrigation with 2 % sodium hypochlorite solution. After drying the root canals with paper points a calcium hydroxide based cement was injected into the root canals. Complete filling of the canals was achieved by the use of lentulo-spiral fillers on a slow speed handpiece. Lastly the canals were obturated with gutta percha points utilizing a lateral condensation technique. The gutta percha filled canals were then sealed using a light cured glass ionomer after which the coronal openings were sealed with a light cured compomer. (HOLMSTROM, 2013)

## Results

In 2013 the biopsied hyperplastic mass was diagnosed as a peripheral odontogenic fibroma by histopathology. Post-operative radiographs taken immediately after the procedure in 2015 satisfied the first stage of post-operative assessment. At eighteen months post operatively the animal was anaesthetised for an unrelated medical problem. An oral examination was performed to assess the condition of the teeth: all fillings were intact and no draining sinuses were noted around them; the

gingivae where the gingivectomies were performed was normal, hyperplasia did not recur and the teeth appeared stable on application digital pressure. Dental radiographs were not performed at eighteen months post operatively; but were performed during a recent scheduled health assessment in 2017 (at twenty four months postoperatively). There was no radiographic evidence of periapical pathology affecting the mandibular and maxillary canines or the maxillary incisor.

## Discussion

Dental disease is a common problem in captive wildlife however there is paucity of data reported in the literature as advanced periodontal examination and procedures are seldom performed (GLATT, *et al*, 2008, FOWLER, 1986). Dental disease has a reported overall prevalence of 35 % in captive jaguars in the USA, with prevalence's in adult and geriatric animals of between 41 % and 52 % respectively; of which canine tooth fractures are the most commonly encountered dental pathological condition requiring intervention (HOPE & DEEM, 2006).

Due to limited opportunity to make routine clinical assessments in zoo species, oral and dental examination and prophylaxis should form part of the examination procedure during any opportunistic intervention. In cases where further dental intervention is indicated, e.g. endodontic surgery, there are problems relating to availability of specialised materials, specialised equipment and, depending on clinical experience of the attending veterinarian, specialised medical or surgical assistance. (FOWLER, 1986).

The principles of periodontal examination, diagnosis and treatment in carnivores are identical to those commonly performed in companion animal species, the model species being the domestic dog (ALBUQUERQUE, *et al*, 2012). The first-line of non-surgical treatment for mild periodontal disease in carnivore species is subgingival and supragingival scaling (manual or mechanical), polishing and sulcar lavage under general anaesthesia (NIEMIEC, 2008). Invasive surgical procedures includes root canal treatment and tooth extraction, and are indicated for treatment of teeth fractures (HOLMSTROM, 2013).

When the vitality of the pulp is compromised, the potential for complications, especially infection and abscessation, is substantial. Ignoring the problem, particularly if it does not seem to bother the patient, can lead to further dental problems, tooth loss as well as systemic ramifications (PAVLICA, *et al*, 2008). The option of extracting the affected tooth is preferable to inaction, with training and experience a clinician can offer the option of retaining a tooth that is sound for function and aesthetics. When RCT is performed under aseptic conditions and using recognised clinical principles, the success rate is 95 % in dogs based on veterinary literature (KUNTSI-VAATTOVAARA, *et al*, 2002). The presence of a periapical radiolucency, a necrotic pulp, and external root resorption of the treated tooth can influence the outcome (KUNTSI-VAATTOVAARA, *et al*, 2002). Follow-up radiographs should be taken routinely to ensure the long term success of the treatment performed and can be scheduled at such time that a skilled endodontic clinician is available in case treatment failure is suspected and further intervention indicated.

Although there are absolute indications for extractions, i.e. when no other treatment option exists, it is not always recommended when alternative, less invasive options are available, i.e. RCT. Extraction of canine teeth is widely performed in small animal practice but even then the procedure is often time consuming and problematic. (GORREL, *et al*, 2013) The main concern in wild animals is provision of aftercare of the patient due to high possibility of suture dehiscence resulting in failure of the mucoperiosteal flap. Furthermore, an immobilisation of the patient will be required after 7-14 days to evaluate the healing status of the extraction site. If at that stage a problem is encountered, prompt surgical intervention is indicated. This can be a problem when there is limited access to a dental

clinician. Therefore endodontic therapy is the preferred treatment of complicated crown fractures of canine teeth when encountered in free ranging or captive wild animals.

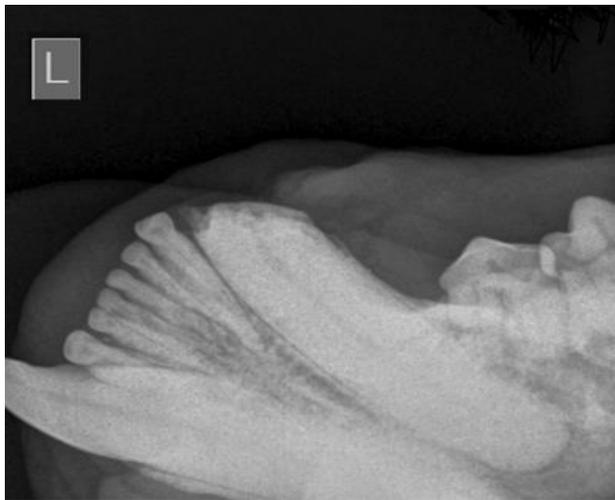
The limitations to engage in advanced periodontal surgical prophylaxis are as relevant to zoological medicine today as when first described by Fowler in 1986. The extended period between this initial recommendation and eventual endodontic intervention to treat the canine fractures in this Jaguar is not an unusual problem encountered when practicing zoological medicine.



*Fig. 1: Initial presentation (15<sup>th</sup> April 2013). Left maxillary and left mandibular canine fractures. Note extensive hyperplasia of gingivae along the maxillary arcade (© Al Ain Zoo, UAE).*



*Fig. 2: Preoperative rostral oblique radio-graph of the left maxilla. Note canine tooth fracture and calcification of hyperplastic gingival tissue (© Al Ain Zoo, UAE).*



*Fig. 3: Preoperative radiograph of left mandibular canine fracture (© Al Ain Zoo, UAE).*



*Fig. 4: Postoperative maxillary oblique radio-graph. Note obturation of 3<sup>rd</sup> maxillary incisor and maxillary canine. (© Al Ain Zoo, UAE).*

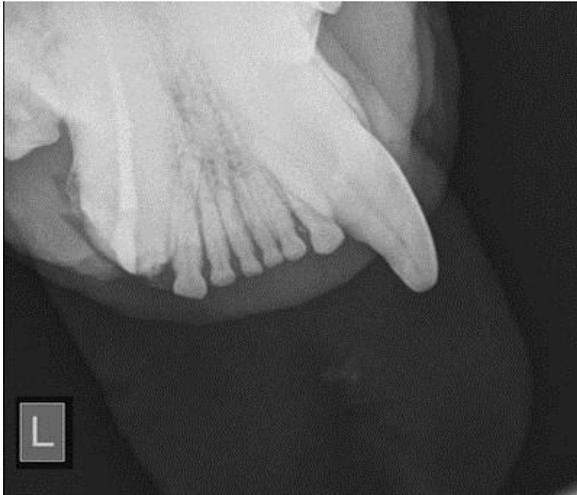


Fig. 5: Postoperative radiograph of mandible. Note obturation of left mandibular canine (© Al Ain Zoo, UAE).



Fig. 6: Photograph 21<sup>st</sup> November 2016. Stable PD evident at maxillary canine tooth. Epulis present though not as extensive in comparison to initial presentation (© Al Ain Zoo, UAE).



Fig. 7: 24 month postoperative radiograph of left maxillary canine and left maxillary incisor teeth fracture: no pathological radiographic findings (© Al Ain Zoo, UAE).



Fig. 8: 24 month postoperative radiograph of left mandibular canine teeth: no pathological radiographic findings (© Al Ain Zoo, UAE).

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## UNILATERAL RENAL CLEAR CELL CARCINOMA IN A VERVET MONKEY (*CERCOPITHECUS AETHIOPS*)

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Primary renal carcinomas occur very rarely in veterinary medicine. Renal cell carcinomas (RCC) has been reported in dogs, cattle and more rarely in cats and pigs. A 16-year-old male vervet monkey (*Cercopithecus aethiops*) in the Uchi Park Zoo, Gwangju, Republic of Korea was found dead without apparent clinical symptoms before death except anorexia and mild lethargy. At necropsy, there was moderate ascites, and multifocal nodules were found in the mesentery, large intestine, spleen and lungs. The masses were solid in consistency with smooth cream coloured cut surfaces. The right kidney was solid and whitish without distinction between the cortex and the medulla, and some cystic areas were observed in both kidneys.

Histopathologically, massive destruction of the tubular structures with intact glomeruli was observed in the kidney. Tubular epithelial cells consisted of vesicular, pleomorphic, usually round with void to spindle shaped nuclei, vacuolated cytoplasm and prominent nucleoli. A lot of vascular and necrotic stroma was found. Multiple sheaths of pleomorphic tumour cells were detected in the lung parenchyma. Immunohistochemically, tumour cells strongly reacted to cytokeratin and vimentin. Grossly, histopathologically and immunohistochemically, the tumour was diagnosed as a RCC, clear cell variant type. To the best of the authors' knowledge, this is the first case of renal clear cell carcinoma with multiple organ metastasis in a vervet monkey. This subject is supported by Korea Ministry of Environment (MOE) as "Public Technology Program based on Environmental Policy (No. E416-00021-0602-0).

## CHANGES IN KIDNEY AND LIVER BIOCHEMISTRY DURING LONG-TERM ANAESTHESIA IN EUROPEAN ROE DEER (*CAPREOLUS CAPREOLUS*)

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Male European roe deer (*Capreolus capreolus*, ten individuals examined twice in January and May 2015, to see a potential seasonal effect) were anaesthetised for a different study and advantage was taken to measure kidney parameters throughout anaesthesia. Since roe deer meat potentially may go into the food chain, the anaesthesia was only conducted with Ketamine (6 mg/kg, Ketamin 10 %, bela pharm, 49377 Vechta, Germany) plus Xylazine (4 mg/kg, Rompun, Bayer, 51368 Leverkusen, Germany), delivered intramuscularly by a blowpipe. For the maintenance, half the dose of Ketamine plus Xylazine was administered every 20 minutes, intramuscularly. The animals were in anaesthesia for 130 minutes. During that time intra-tracheal oxygen (flow 1,5L/min) and an intravenous infusion (flow 30 mL/kg/h) were installed.

The first blood sample was taken within 8 and 12 minutes after darting, the second sample on 120 minutes after darting. Recovery was unremarkable and occurred 14-29 minutes after 0.4 mg/kg Atipamezol injection. Creatinine increased significantly from  $1 \pm 0.15$  to  $1.7 \pm 0.3$  mg/dL (median  $\pm$ SD). Biliary-urinary nitrogen increased from  $26 \pm 4.7$  to  $30 \pm 3.31$  mg/dL. ALT increased from  $36.5 \pm 5.7$  to  $50 \pm 11.5$  U/L, Alkaline Phosphatase from  $47 \pm 12.9$  to  $85 \pm 21.7$  U/L, and AST from  $97.5 \pm 27$  to  $192 \pm 51.9$  Units/L.

As expected with this high dose of alpha 2 agonists, the blood pressure was very high shortly after i/m injection with peak levels of 208/142 (systolic/diastolic), which decreased slightly throughout anaesthesia. The kidney and liver parameters increased significantly during anaesthesia, even though the animals received intravenous fluids showing once again the importance of fluid-therapy during anaesthesia. The demand for changes in the legislative to accept a more balanced and modern anaesthesia in wildlife which is potentially going into the human food chain is high. Unfortunately, Ketamine plus high levels of Xylazine are still widely used for wildlife anaesthesia protocols, especially for those going into the food chain, but also in zoos.

**SEASONAL ALOPECIA IN A POLAR BEAR (*URSUS MARITIMUS*) FEMALE**PAINER J<sup>1</sup>, TUJULIN E<sup>2</sup>, WEBER AM<sup>2</sup>, DAVINA I<sup>3</sup>, WELLE M<sup>4</sup>, OLSSON A<sup>2</sup>, BRUNBERG S<sup>2</sup>

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A female polar bear was presented with recurrent seasonal alopecia from September to February. The first symptoms start in August with pruritus and stereotypies, and hypotrichosis develops in October. In January the hair loss reaches a peak and is characterised by symmetrical alopecia with partly bald areas, which are most pronounced on the legs, the flanks, the back, the breast and forehead. From February onwards, the hair starts to regrow and the bear has a normal fur from April to August (observed from 2010 – 2017).

Diagnostics included skin biopsies, exclusion of ectoparasites and fungi, haematology, serum biochemistry, hormone - (progesterone, estrogen, testosterone, cortison, trijodidthyronin, thyroxin), vitamin and heavy metal status. All parameters were within published references or negative. Standard therapies, like repeated administration of ivermectin, Vitamin ADE and fish oil were given, without any benefits.

Histological examination of skin biopsies from completely alopecic regions revealed a complete absence of hair follicles, a moderate perivascular, mainly lymphocytic and plasmacellular infiltrate, a partially parakeratotic and partially orthokeratotic hyperkeratosis associated with a moderate epidermal hyperplasia and hyperpigmentation. The animal was treated with 0.4 mg/kg/day Oclacitinib (Apoquel, Zoetis, Switzerland) from July to February. Apart from a period of two weeks in September, no itching and stereotypies could be observed, which is a big success for the animals' welfare. For the first time, she had a significantly better fur throughout the winter, although symmetric thinner hair quality was seen in some areas, while no areas were completely bald anymore. The alopecia seems to be the consequence of an atopic dermatitis-like inflammatory reaction of yet unknown cause.

## FOUR CASES OF FATAL ACUTE TOXOPLASMOSIS IN FENNEC FOX (*VULPES ZERDA*)

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*Toxoplasma gondii* is a zoonotic, obligate intracellular protozoan parasite capable of infecting all warm-blooded animals. Four acute disease cases of fatal toxoplasmosis in fennec fox (*Vulpes zerda*) have occurred in the Al Ain Zoo collection in the United Arab Emirates since 2015. The first case was an adult female kept alone, that was sick and off-food for two days presenting bilateral nystagmus and blindness. The animal was euthanised on welfare grounds, and a complete post-mortem examination was performed and samples were collected for further studies. The histopathological study revealed a multifocal granulomatous meningoencephalitis and protozoal cysts in the brain. qPCR detection of *T. gondii* was performed by using the commercial ToxGon dtec-qPCR Test kit from Genetic PCR Solutions™ confirming the presence of *T. gondii* DNA in samples collected from the brain.

A new pair of animals was introduced, and a second case occurred one year later in their offspring. The juvenile fox was found dead without any prior clinical signs. Again, a complete post-mortem examination was performed and samples were collected for further studies. Macroscopic lesions were found in liver, spleen and heart. Histopathology confirmed a systemic disease and the presence of protozoal organisms within the myocardial lesions. Real-time PCR analysis in house and genetic results from an external institution identified a highly virulent type I strain of the three types of *T. gondii*.

Recently, two juveniles from a new litter of four animals died after initially presenting respiratory signs. Severe meningeal congestion was evident during subsequent post mortem examinations and *T. gondii* DNA was detected by real-time PCR in the brain tissue from both animals. Histopathological analysis confirmed the presence of a systemic protozoal infection in both cases, and immunohistochemical staining confirmed the presence of *Toxoplasma* antigen in brain, lungs, spleen and myocardial lesions. Surveillance studies in the Zoo animal collection, and on feral animals and food are ongoing to determine the source of *Toxoplasma* infection.

## SQUAMOUS CELL CARCINOMA OF THE *SINUS INFRAORBITALIS* IN A JUVENILE WHITE STORK (*CICONIA CICONIA*)

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### Summary

A case of a squamous cell carcinoma at the head of a White Stork (*Ciconia ciconia*) is described. First it looked like a sinusitis of the *Sinus infraorbitalis* and was treated accordingly but it was fast growing. After excluding an abscess and performing X-rays the stork was euthanised due to the dimension of the mass and inoperability. On necropsy, the mass filled up the whole nasal cavity, was filled with caseous material and diagnosed as a squamous cell carcinoma. Additionally, a possible secondary *Escherichia coli* infection of the neoplasia was found.

### Introduction

Squamous cell carcinomas at the head of birds are most prevalent at the mucocutaneous junctions. This kind of tumours is in most case locally invasive but rarely metastasising. A surgical attempt is the most promising whereby a complete excision is not often accomplishable (LIGHTFOOT, 2006). Additionally radiation and photodynamic therapy is reported but with limited success (MANUCY *et al.*, 1998; ROSENTHAL *et al.*, 2001; SUEDEMEYER *et al.*, 2001).

### Case

A juvenile White Stork (*Ciconia ciconia*) was submitted to the Wildlife Rescue and Conservation Centre due to weakness and inability to fly. During the first clinical examination a 4x4cm big mass with a 1cm long ulceration was found in the region of the left *Sinus infraorbitalis*. The left eye was without pathological findings. Thus, a traumatic sinusitis was suspected and treated orally with 10 mg/kg body weight Enrofloxacin (Baytril® 2,5 %, Bayer Vital GmbH, Leverkusen, Germany) per day, 0,5 mg/kg body weight Meloxicam (Metacam®, Boehringer Ingelheim Vetmedica GmbH, Ingelheim/Rhein, Germany), and two times a day with an antibiotic eye ointment (Floxal®, Dr. Gerhard Mann chem.-pharm. Fabrik, Berlin, Germany). While hospitalised the stork exhibited a good general condition and an appropriate food intake. After five days of treatment no improvement was observed and further diagnostics were needed. The radiographic examination uncovered a complete involvement of the left part of the head. Additionally, the *Sinus infraorbitalis* and the nasal cavity were infiltrated. Moreover, the left sclerotic ring was withdrawn. At this time the mass had increased in size to 7x7cm and covered the left eye completely. It was ulcerated and bled. Furthermore, it bulged out to the oral cavity and

deformed the hard palate. The mass was opened by making an incision with a scalpel under general anaesthesia with Isoflurane (1-4 Vol.-%, Isofluran-CP®, CP-Pharma GmbH, Burgdorf, Germany). But no abscess was found in the *Sinus infraorbitalis*. The mass consisted of a friable tissue with little necrotic and purulent parts. Therefore, the mass was classified as inoperable, leading to euthanasia of the stork.

### Results of further diagnostics

A subsequent necropsy confirmed friable tissue with little necrotic and purulent parts.. Macroscopically the other organs were without pathological findings and no metastases were observed. In the histopathologic examination of formalin fixed, paraffin embedded and routine haematoxylin and eosin stained tissue samples the mass was diagnosed as squamous cell carcinoma. Swabs were collected from the purulent parts and submitted for routine microbiologic examination, culturing a high number of enrofloxacin resistant *Escherichia coli*.

### Discussion

The presented case is to our knowledge the first observation of a squamous cell carcinoma in the infraorbital sinus of a White Stork. Until now a squamous cell carcinoma in White Storks is only reported on the upper beak of an immature stork (LOPEZ-BECEIRO *et al.*, 1998). Squamous cell carcinoma is a malignant tumour of squamous cells predominantly of the upper gastrointestinal tract but also of nasal sinuses and especially the skin (SCHMIDT *et al.*, 2015). These tumours are extremely locally invasive and are rarely able to metastasize for example to the lung (LIGHTFOOT, 2006; LAURA *et al.*, 2016). As it was observed in this case many squamous cell carcinomas in birds have ulcerated surfaces and caseous material can be found within the mass (SCHMIDT, 1992; DIAZ-FIGUEROA *et al.*, 2006; SCHMIDT *et al.*, 2015). Another characteristic feature of this neoplasia in birds are infiltrative cords of moderately undifferentiated to poorly differentiated cells that form central cores of laminated keratin (REAVILL, 2004; DIAZ-FIGUEROA *et al.*, 2006; SCHMIDT *et al.*, 2015). As the tumour described in this case the squamous cell carcinoma in a White Stork reported by LOPEZ-BECEIRO *et al.* (1998) was growing rapidly and causing a deformity of the hard palate. Other possible neoplasia affecting the infraorbital sinus in birds are fibrosarcoma, adenoma, papilloma, liposarcoma, and lymphosarcoma (BAUCK, 1992; GRAHAM *et al.*, 2003; OLSEN, 2003).

Furthermore, in the present case *E.coli* was detectable in the mass, because of a secondary infection of the primary neoplasia or as the reason of an initial sinusitis. *E.coli* is described as a causative agent of sinusitis in birds (SCHMIDT *et al.*, 2015). It is worth considering that squamous cell carcinomas tend to develop on sites with chronic irritation (TURREL *et al.*, 1987). The evidence of a visible swelling in the area around the eyes in a wild bird leads to the suspicion of a traumatic or an infectious etiology. In this case a traumatic injury was supposed because of the small size of the wound and swelling. Traumatic injury is one of the most frequent causes of death in young storks (OLIAS *et al.*, 2010). Traumatic causes of a periocular swelling can be lacerations, haemorrhages, and abrasions (BAYÓN DEL RIO, 2016). After the inefficient treatment an abscess in the sinus was suspected. Possible infectious etiologies for sinus distension in storks include aspergillosis, mycoplasmosis, mycobacteriosis, avian paramyxovirus, and chlamydiosis (OLIAS *et al.*, 2010; KALETA and KUMMERFELD, 2012; NORTON and WHITESIDE, 2015; MÖLLER PALAU-RIBES *et al.*, 2016). In this case no indication of infectious agents were found on the histopathologic examination and there was no fungal growth in the microbiologic examination. For surgical debridement of the suspected caseous mass a sinusotomy is the recommended therapy (ARCA-RUIBAL, 2016).

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## OCCURRENCE OF VERMINOUS GRANULOMAS IN THE LUNG OF A POLECAT (*MUSTELA PUTORIUS*) INDUCED BY *FILAROIDES MARTIS*

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### Summary

This case report describes a severe lungworm infection in a free-ranging polecat (*Mustela putorius*) with multiple nodular granulomas in the lung, which were detectable by radiographs. The polecat exhibited a bad general condition and emaciation after a suggested traumatic spinal cord injury. The polecat was euthanised because of its general condition and hind limb paraplegia, and a necropsy was performed. Histopathological examination revealed a lungworm infection, the nematode species was identified as *Filaroides martis* based on its morphology and genetic results.

### Introduction

The polecat (*Mustela putorius*) is a medium-sized carnivore mustelid, which feeds on a wide variety of prey, e.g. small mammals, birds, amphibians, reptiles, and insects (COOPER, 2003; KOLLIAS and FERNANDEZ-MORAN, 2015). Hence, a wide range of helminths are reported in polecats in the literature (SHIMALOV and SHIMALOV, 2002; TORRES *et al.*, 2008; KOLLIAS and FERNANDEZ-MORAN, 2015). Thereof, *Crenosoma* spp., *Eucoleus aerophilus*, *Filaroides* spp., and *Aelurostrongylus* spp. colonize the lung parenchyma and the airways, whereas *Diriofilaria immitis* and *Angiostrongylus* spp. settle in the pulmonary arteries (SASAI *et al.*, 2000; SHIMALOV and SHIMALOV, 2002; TORRES *et al.*, 2008; KOLLIAS and FERNANDEZ-MORAN, 2015; KRETSCHMAR, 2016).

### Case

A free-ranging adult female polecat (*Mustela putorius*) was admitted to the Clinic for Small Mammals, Reptiles, and Birds of the University of Veterinary Medicine Hannover, Germany due to an observed paraplegia. During the clinical examination, the polecat showed a bad body condition and apathy as well as fleas and ticks. The hind limbs were paraplegic without any reflexes. To ascertain possible fractures, the polecat was radiographed, whereby multifocal nodular masses with soft tissue density were detected in the thorax. Based on the poor prognosis resulting from the bad general condition, the hind limb paraplegia and the alterations in the lung, the polecat was euthanised. During necropsy, extensive hematomas all over the body were present, especially in the lumbar muscles and the pelvis. The lungs showed multifocal nodular masses with a diameter up to 1cm. Some granulomas exhibited a thin outer membrane with centrally large numbers of worms. The histopathological examination revealed a moderate, multifocal, lympho-histiocytic, partial granulomatous pneumonia with adult and

larval nematodes in the alveolar spaces and peribronchial tissue. The helminths were defined morphologically as the first larval stage of *Filaroides* sp. according to the description by ANDERSON (2000). For the exact species identification, DNA was extracted from isolated worms and analysed by polymerase chain reaction according to CARRENO and NADLER (2003) using primers 391 forward (5'-AGCGGAGGAAAAGAACTAA) and 501 reverse (5'-TCGGAAGGAACCAGCTACTA) for the 5' end of the 28S region and 537 forward (5'-GATCCGTAACCTTCGGGAAAAGGAT) and 531 reverse (5'-CTTCGCAATGATAGGAAGAGCC) for the 3' end of the large subunit rDNA. Subsequent sequencing analysis resulted in a complete homology with *Filaroides* (*F.*) *martis*.

## Discussion

Lungworm infections in polecats are not uncommon and were found recently in about 54 % of investigated polecats in Germany, whereby *F. martis* was the most frequent nematode found in monoinfections but also coinfections with other lungworm species were detectable (KRETSCHMAR, 2016). *F. martis* often occurs in nodules at the hilus of the lung lobes as observed in this case (ANDERSON, 2000). In the described case, however, the nodular alterations were located also in the parenchyma of the lung. Additionally, the nodules were projecting from the lung surface as it is characteristic for *Aelurostrongylus* infection (TAYLOR *et al.*, 2016a). *Aelurostrongylus* (*A.*) infection in polecats was reported by KRETSCHMAR (2016) with no further species differentiation, while in other mustelids *A. pridhami* and *A. falciformis* were identified (TORRES *et al.*, 2001; TORRES *et al.*, 2008). Nodule formation is untypically for *Crenosoma* (*C.*) species in polecats (ANDERSON, 2000), but *C. vulpis* can cause granulomas in dogs and foxes, which are detectable by radiographs (REILLY *et al.*, 2000; PRIEMER, 2001). The most common species in polecats is *C. melesi*, but also *C. taiga*, *C. petrowi*, and *C. schachmatovae* were identified (ANISIMOVA, 2002; TORRES *et al.*, 2008; KRUCHKOVA *et al.*, 2013; NUGARAITÉ *et al.*, 2014; KRETSCHMAR, 2016). *Eucoleus aerophilus* (syn. *Capillaria aerophila*) is another lungworm species found in polecats (TORRES *et al.*, 2008; NUGARAITÉ *et al.*, 2014; KRETSCHMAR, 2016). In this case nematode larvae were seen in lung smears, which is not typical for *Eucoleus* infection, where only eggs are present (TAYLOR *et al.*, 2016b). The infection cycle of *F. martis* includes gastropods as intermediate hosts. After a polecat fed on an infected gastropod, nematode larvae leave the stomach and follow arteries to the lung where they settle in the peribronchial tissue at the lung hilus as observed in this case (ANDERSON, 2000). The diagnosis of a lungworm infection is usually done by coproscopy, where the first larval stages are detectable in the faeces (ANDERSON, 2000). Morphological classification of the larvae reaches the genus level and is carried out based on the specific posterior endings of the first stage larvae (TAYLOR *et al.*, 2016a). In the present case the polecat was euthanised and coproscopy was performed during necropsy. Larvae detected in the faeces were morphologically identical to those in the lung. Infected polecats can be treated with levamisole (DINNES, 1980).

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**CLINICAL AND HISTOPATHOLOGICAL FEATURES OF TRYCHOPHYTON SPP. INFECTION IN TWO WILD RACCOON DOGS (*NYCTEREUTES PROCYONOIDES*)**RHIM H<sup>1,2</sup>, LEE D<sup>2</sup>, BAE JE<sup>1</sup>, KIM HC<sup>1</sup>, HAN JI<sup>1,2</sup>

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Two rescued wild raccoon dogs (*Nyctereutes procyonoides*) showed multiple round-to-oval encrustations with hypotrichosis on the shoulder, armpit, and abdomen. Deep-skin scraping and microscopic examination revealed inflammation and several fungal conidia with septated hyphae. Plucked hairs from each lesion were inoculated and cultivated on Sabouraud dextrose agar. After 10 days, a fungal colony with a white-to-yellow velvety irregular shape and brown pigmentation in the center appeared, suggesting the presence of dermatophytes. After DNA extraction, polymerase chain reaction targeting the internal transcribed spacer (ITS) region, including ITS1 to ITS4, was performed and direct sequencing helped identify the isolated fungus as *Trichophyton* sp. (but not *T. mentagrophytes*). Pyogranulomatous-to-eosinophilic dermatitis with intralesional fungal growth within the hair follicles was found on histopathological examination. Characteristic parakeratotic hyperkeratosis with intracorneal pustules and encrustations was also seen. Dermatophytosis is known to produce typical clinical signs, such as well-circumscribed alopecia, thickened epidermal layer, and dry scale, in domestic dogs. The raccoon dog is a canid very similar to the domestic dog. Macroscopic and histological changes of dermatophytosis in these two raccoon dogs, however, were different to those typically seen in domestic dogs. Therefore, in raccoon dogs, not only scabies infection but also dermatophytosis should be considered when they show crusted and scaly skin. This study is supported by the Korea Ministry of Environment (MOE) as a "Public Technology Program based on Environmental Policy" (No. 2016000210002).

## EVALUATION OF KETAMINE AND MEDETOMIDINE AS AN ANAESTHETIC REGIME FOR REMOTE CHEMICAL IMMOBILIZATION IN DANUBE DELTA FREE RANGING FERAL HORSES

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The purpose of this study was to evaluate the physiological parameters of 91 free ranging feral horses (*Equus caballus*) remotely immobilised with a combination of 775 mg Ketamine and 30 mg Medetomidine, using a single disposable 6 ml dart. During anaesthesia, heart rate, respiratory rate, temperature, capillary refill time and hemoglobin oxygen saturation were evaluated. For 8 horses, arterial blood samples were analyzed to evaluate the blood gases, acid-base status and hematologic variables. Target horses presented a broad variability of body weight (range 80 to 375 kg) and age (range 1 to 18 years old). The shortest induction time of  $\bar{x} = 3.6$  min was achieved for animals less than 200 kg, while the horses between 200 and 300 kg and those over 300 kg, became recumbent in  $\bar{x} = 8.18$  min, respectively  $\bar{x} = 10.56$  min. Ten horses required supplemental doses of Ketamine intravenously ( $\bar{x} = 1.35$  mg/kg) secondary to an inadequate anaesthetic plane. For financial reasons, only 58 immobilisations were reversed with  $\bar{x} = 0.1$  mg/kg Atipamezole intravenously. Increased respiratory rate (> 20 breaths), heart rate (> 45 bpm) and low SpO<sub>2</sub> (< 90 %) were noted in more than half of the subjects, while increased body temperature (> 39.0°C) was recorded in six animals. Blood gas parameters showed hypoxemia (< 90 mmHg, n = 8), hypercapnia (>45 mmHg, n = 5), high glucose levels (> 134 mmol/L, n = 3), increased blood lactate (> 1.5 mmol/L), TCO<sub>2</sub>, HCO<sub>3</sub> and BE between the sample points, whereas SpO<sub>2</sub> and iCa values were lower. Most horses showed a fully relaxed anaesthesia, smooth recovery, with one (n = 86) or more (n = 5) attempts of standing. A longer induction time was correlated with shorter recumbency time. On average, remobilised horses were laterally recumbent for  $\bar{x} = 66.82$  min while those without Atipamezole were recumbent for  $\bar{x} = 67.69$  min. Most recoveries were uneventful, with exception of one male that showed signs of left radial nerve neuropathy and one mare with signs of exertional myopathy. In conclusion, a duration of 60 minutes of anaesthesia was sufficient for decreasing the Ketamine and Medetomidine plasma levels, the systemic compromises during recumbency time being tolerated in healthy animals.

## RENAL INSUFFICIENCY IN CAPTIVE WEST INDIAN MANATEE (*TRICHECHUS MANATUS MANATUS*) – A CASE STUDY

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The Manatee Reintroduction Project led by National Park of Guadeloupe aims to restore the population of West Indian manatees (*Trichechus manatus manatus*) in Lesser Antilles. In August 2016 two captive born manatees were transported from Singapore Zoo to Guadeloupe. Both were subjected to the routine health assessment on the day of departure and seven weeks after arrival. Translocation entailed a 34-hour flight which both manatees managed without complications. The manatees were related and had 6 and 7 years respectively. The first assessment revealed a lean condition of one manatee with concave abdomen. Second capture of this animal showed a weight loss of 28 % of the previous body weight. Urea and creatinine increased 3.8 times and 2 times above normal respectively (HARVEY *et al.*, 2007). The animal died 48 hours later. Histopathology indicated chronic nephritis with interstitial fibrosis, glomerular sclerosis and mineralisation. SDMA (symmetric dimethylarginine) is a biomarker known to be more specific to kidney function than creatinine and used in early renal insufficiency diagnosis. Retrospective analysis of SDMA from the evaluation done when loading demonstrated a subclinical renal insufficiency. Repeated analysis from two days before decease showed a double increase of the value, which, together with other pathological findings, confirmed an exacerbation to acute renal insufficiency. Creatinine and urea are known to increase with anorexia. Therefore SDMA has a potential as a useful test in the routine health screening of manatees and may be especially useful when relocating manatees.

## **NEW FEEDING STRATEGIES FOR CAPTIVE BARBARY MACAQUES (*MACACA SYLVANUS*): IMPROVING WELFARE AND SOCIAL LIFE**

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In some zoos food for primates is chopped into small pieces. To provide whole food might be enriching as it may promote natural species-specific behaviours, whereas to chop food might lead to an increased risk of bacterial contamination and food wastage. This study aimed to assess the effects of food management on the behaviour of a captive colony of thirteen Barbary macaques (*Macaca sylvanus*). Three feeding set-ups were compared: the Baseline with chopped food; the Two-Whole with whole food items (two pieces of each type of fruit and vegetable per subject); the One Whole with whole food items (one piece for each type of fruit and vegetable per subject). Twenty 10-minute sessions were run per subject and per set-up. Continuous focal animal sampling method was used to collect individual and social behaviours. In particular, the dominance hierarchy was evaluated for each set-up to assess the effects of the food management on the macaque social relationships. Data were analysed using non-parametric tests and significance level was set at  $P < 0.05$ . Results suggest that presenting whole food was enriching for this colony by promoting individual and social species-specific behaviours. Affiliative behaviours, especially grooming, were performed significantly more in the whole food set-ups than in the baseline. Furthermore, the hierarchy did not vary significantly among set-ups and aggressive behaviour decreased. In conclusion, to provide food to Barbary macaques in a natural way - whole food - seems to be a valuable tool to assist in managing their social relationships and welfare.

## UNSOLVED DIFETHIALONE INTOXICATION IN A SOUTHERN ROCKHOPPER PENGUIN (*EUDYPTES CHRYSOCOME*) AND A BLACK STORK (*CICONIA NIGRA*)

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Difethialone is an anticoagulant that is used as a rodenticide. It is highly potent and a single dose can lead to uncontrolled bleeding and death. In Germany it is only allowed to be used by pest management professionals.<sup>1</sup>

In March 2016, a 3-year-old Southern rockhopper penguin (*Eudyptes chrysocome*) was found dead without any previous clinical signs in a zoo in Southern Germany. A month later, in an approximately 80 km distant zoo, an adult black stork (*Ciconia nigra*) was also found dead without any previous clinical signs. Pathological, bacteriological, parasitological and histological examinations were conducted on both birds following standard protocols. Toxicological examinations were then performed on liver samples using high performance liquid chromatography (HPLC) by the institute of pharmacology, toxicology and pharmacy, LMU Munich.

Necropsy revealed uncoagulated blood in the organs and toxicological examination revealed residues of difethialone in both birds.

The source of the difethialone is unsolved. Difethialone was not used in the zoos and the dead birds were kept in aviaries with other birds, which did not show any sign of intoxication. In the case of the Southern rockhopper penguin, contact with visitors was not possible due to the housing design. Both the Southern rockhopper penguin and the black stork had a lot of fish in their digestive tract. Intoxication via contaminated feed or water cannot be ruled out, although the water of the penguin enclosure was tested negative for residues of difethialone.

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## **SYMPTOMS OF AUJESZKY'S DISEASE AND PATHOLOGIC ANATOMICAL CHANGES IN INTERNAL ORGANS OF A BOBCAT**

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In a private zoological collection a four-year-old bobcat became ill and then died. Death occurred at the end of the second day of illness. Clinical signs of disease were fearfulness and anxiety, loss of appetite, low-grade fever, dyspnea and hoarseness, progressive neurological lesion sites, scratched gums, excessive salivation, unsteady gait and paralysis. After the immobilisation by means of flying syringes using a blowgun, a clinical examination of the bobcat and temperature measurement were carried out, the mouth was examined, saliva was sampled for immunological investigation and blood was sampled for morphological and biochemical investigations.

The bobcat's blood showed significant leukocytosis, neutrophilia and lymphopenia, which involved the development of an infectious process. When serum biochemical studies showed hyperglobulinemia, serum alkaline phosphatase and cellular enzymes ALT and AST increased two or three times, which is characteristic of hepatitis and pancreatitis, blood glucose increased five-fold due to the enhanced glycogen breakdown and the excitability of the cerebral cortex increased (sympathetic hyperglycemia syndrome). Polymerase chain reaction (PCR) confirmed the diagnosis - Aujeszky's disease. Treatment in the bobcat was ineffective. The autopsy revealed signs of multiple organ pathology: acute purulent encephalitis, pulmonary edema, catarrhal gastroenteritis; degeneration of parenchymal organs.

## FULMINANT HEPATITIS INDUCED BY HERPESVIRUS IN A COMMON WOMBAT (*VOMBATUS URSINUS*)

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A male 2-year-old Common wombat (*Vombatus ursinus*) suddenly began showing symptoms of inappetence, weight loss, polyuria and polydipsia. Blood collection revealed elevated liver enzymes, glucose, fructosamine and CK values, low WBC count and elevated serum molybdenum and copper values. Abdominal ultrasound showed hyperechoic and dissociated hepatic tissue, as well as dilated intestinal loops. Despite supportive therapy, the animal died three days after the onset of clinical signs. On necropsy, edematous lungs, cardiac muscle pale and friable, dilated and gas-filled intestinal loops, pale kidneys, splenomegaly and hepatomegaly, with extremely fragile hepatic tissue, were found. Histopathology revealed necrotizing, non-suppurative myocarditis with areas of myocardial fibrosis, type II crescentic glomerulonephritis and focal hepatic necrosis with mononuclear infiltration and complete structural dissociation. Liver sections showed scattered intranuclear viral inclusions within the necrotic areas, and anti-herpesvirus immunoperoxidase stain evidenced strong nuclear staining. Within these zones, residual hepatocytes had enlarged "ground glass" nuclei with margination of chromatin. These findings could be consistent with fulminant hepatic necrosis due to herpesvirus. Transmission electron microscopy revealed hexagonal 100 - 110 nm in diameter capsids within distorted nuclei of hepatocytes, in a central zone immediately surrounded by a thick band of dense chromatin or in a mixture of electron-dense material, marginated against the nuclear membrane, but not in the extracellular space. A nested Pan-herpesvirus PCR showed a positive fragment that will be sequenced to confirm the result. To the best of our knowledge, this is the first report of a herpesvirus induced hepatitis in the Common wombat outside his native area.

## TO FEED OR NOT TO FEED? RUMEN MUCOSA HEALTH IN HUNTED ROE DEER (*CAPREOLUS CAPREOLUS*) WITH AND WITHOUT SUPPLEMENTAL WINTER FOOD

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Supplementary feeding of wild ruminants is used to reduce winter mortalities and levels of agricultural or forestry damage. However, it has been proven to potentially induce health problems, such as rumen acidosis. Supplemental winter feeding of free-ranging roe deer (*Capreolus capreolus*) has indeed been shown to influence forestomach pH, but it is unclear whether this is associated with pathological effects on mucosal and overall health. We compared rumen mucosa histology between three groups (A-C), of 10 hunted roe deer (A: hunted in the summer, unsupplemented, mean forestomach pH  $5.5 \pm 0.1$ ; B: hunted in the winter, unsupplemented, pH  $6.0 \pm 0.1$ ; C: hunted in the winter, supplemented, pH  $5.1 \pm 0.1$ ). Scoring the degree of hyperkeratosis, parakeratosis, inflammation, epithelial sloughing, epithelial living layer vacuolisation, *stratum corneum* ballooning and *stratum granulosum* degeneration, only differences in hyperkeratosis, parakeratosis and inflammation were significant between groups. Hyper- and parakeratosis scores were higher in group C and at their lowest in group B. Group A had intermediate scores, but the summer animals with the lowest pH had scores similar to the scores in group C. There was a significant negative correlation between the individual forestomach pH values and the level of hyper- and parakeratosis. *Stratum corneum* ballooning degeneration and epithelial living layer vacuolisation did not yield significant correlations, hence their use and an estimate for rumen health cannot presently be advocated in this species. The results indicate that the supplemental feeding regime used in this study induces pH and mucosa states similar to those observed under natural feeding conditions in summer.

## EUROPEAN AND AMERICAN CLINICAL COOPERATIVES: A NOVEL VETERINARY STUDENT STUDY ABROAD PROGRAMME

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Purdue University's College of Veterinary Medicine (PVM) USA and the University of Veterinary and Pharmaceutical Sciences Brno (UVPS) CZ entered a memorandum of understanding in 2008. The focus was to develop clinical cooperatives with visitor exchanges hosting faculty and veterinary students. Several 4<sup>th</sup> and 5<sup>th</sup> year UVPS veterinary and food hygiene students visited Purdue through their Erasmus charter for a month annually and rotations were included through PVM and the College of Agriculture.

The PVM's inaugural study abroad course for credit became a 3 week faculty led programme in Exotic Pet Medicine and Surgery and participation in Zoological and Conservation Medicine. Twenty-nine veterinary students, who completed their first or second year, have participated in this three weeks, three credit course. The heart of the programme remains 10 to 12 days of exotic pet clinics with reptiles, small mammals and birds at UVPS with an annual caseload exceeding 5,000 visits. On site experiences have also occurred with wildlife and care of the collection at Zoo Brno, and tour opportunities with Tiergarten Schönbrunn and Zoo Praha. Networking expanded to include Equine and Camelid services from UVPS. Prof Knotek's joint appointment with the University of Veterinary Medicine in Vienna (VetMedUni) allowed students to participate with avian, reptiles and aquaculture in addition to an active spring wildlife rehabilitation service on site. This has since expanded to include the Messerli – Research Institute and their behaviour studies through the Clever Dog, Corvid and Kea labs and will include small mammals at VetMedUni in 2017.

### Acknowledgements

The authors would like to acknowledge the support of their Rectors, Deans, Department Heads, International Programs offices, colleagues and spouses. In 2015, Prof Knotek and Dr. Jekl became adjunct faculty within the Department of Veterinary Clinical Sciences in the PVM in recognition of their ongoing teaching cooperatives with PVM students.

Purdue University Study Abroad and International Learning grants, Purdue University Study Abroad Initiative scholarships, Indiana VMA, SAVMA Study Abroad scholarships, PVM Spates Pet Bird Endowment and PVM Office of Lifelong Learning have provided funding and assistance that has offset some of the faculty and student costs for this study abroad course while also enhancing UVPS faculty visits to benefit PVM students onsite in West Lafayette. UVPS and VetMedUni faculty have been speakers related to exotic pets and diversity during PVM Continuing Education conferences for veterinarians, technicians and pet owners as well as college lectures and national SAVMA and APVMA symposia for undergraduate and professional students.

## **BRUCELLA MELITENSIS INFECTION IN DORCAS GAZELLES (GAZELLA DORCAS) IN ISOLATED SEMI FREE RANGE ZOOLOGICAL COLLECTION IN THE U.A.E**

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### **Summary**

At private zoo collection in Dubai (UAE), dorcas gazelles have been kept successfully for many years. Populations grew constantly. Group of gazelles of different age showed clinical signs of sickness that includes hygromas of joints pathognomonic for brucella infection. *Brucella melitensis* was isolated. A management programme to cull gazelles with clinical signs and to check the size of gazelle population was implemented.

### **Introduction**

The dorcas gazelle (*Gazella dorcas*) is one of the smallest gazelle species. It is found all over Northern Africa and part of the Middle East. The species is considered as “vulnerable” on the IUCN Red List of Threatened Species (IUCN RED LIST OF THREATENED SPECIES, 2008).

*Brucella melitensis* appears to be a common disease in domesticated bovids (ALTON 1990) and camelids species in the Middle East (KARIM *et al.*, 1979; RADWAN *at al.*, 1983, ISMAILY *at al.*, 1988, AL-KHALAF *at al.*, 1989, WERNERY *at al.*, 1990, AFZAL *at al.*, 1994, ABBAS *at al.*, 2002, RAFAI *et al.*, 2002, WERNERY, 2014) , but is rarely diagnosed in wild bovids (ESSAWY *at al.*,2011, MOLLAH *at al.*,2002, OSTROWSKI S., 2002) and specifically in gazelles under remote isolated conditions (SOARES *et al.*, 2013) or not diagnosed at all (OFFNER *et al.*, 2007).

### **Material and methods**

The group of gazelles of different age was presented for binocular examination. The 300 ha enclosure of semi-arid area had no grazing vegetation and gazelles designed herbivores pellets and alfalfa grass was supplied on the daily basis. The gazelles had access to the fresh water.

Binocular examination of a group of gazelles showed that some of them displayed the following clinical signs: reluctance to walk, staring hair coat, general ill thrift and “hygromas” - enlarged, swollen carpal, tarsal, and phalangeal joints. Hygromas were of different sizes and occurred uni- or bilaterally. Four animals of the affected gazelles, two females and two males which showed obvious sign of sickness were culled and submitted to the Central Veterinary Research Laboratory (CVRL, Dubai) for investigation. Blood was collected for serological examination. All four gazelles showed carpal/ tarsal hygromas and two males displayed symmetrical testicles with no sign of enlargement. All four were also RBT positive, and three out of four were Real Time PCR positive for *Brucella melitensis*. From the two PCR positive gazelles pus was collected, which had accumulated in the tarsal joint from which high numbers of *Brucella melitensis* were isolated.

## Discussion

Brucellosis caused by *Brucella melitensis* is an important and dangerous disease for production animals and, being a zoonosis, even for humans (WORLD HEALTH ORGANIZATION (WHO) & FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS (FAO), 1986). *Brucella melitensis* infection in the representative of wild bovids is currently limited to a number of gazelles and antelopes species, such as Arabian oryx (*Oryx leucoryx*) (OSTROWSKI *et al.*, 2002), Arabian Neumann's gazelle (*Gazella enlargeri*) (ESSAWY *et al.*, 2001), Sable antelope (*Hippotragus niger*) (GODFROID *et al.*, 2013), (AFRICAN WILDLIFE DISEASE, 2016), Impala (*Aepyceros malampus*) (SCHIEMANN and STAAK, 1971) and Dorcas gazelle (*Gazella dorcas*) (KAMEL, 1950).

The main reservoirs of the *Brucella melitensis* in the Arabian Gulf countries seem to be cattle, sheep, goats and camels (KARIM *et al.*, 1979; RADWAN *et al.*, 1983, ISMAILY *et al.*, 1988, AL-KHALAF *et al.*, 1989, WERNERY and WERNERY, 1990, AFZAL and SAKKIR, 1994, ABBAS and AGAB, 2002, WERNERY, 2014), However in a remote location direct spill-over from domesticated animals to the wild life is not expected, especially because in our case, there were no sheep and goats farm in the vicinity. Given the limited occurrence of *Brucella melitensis* in gazelles species the prevalence is unknown, it is assumed that the infection may be contracted from a sources of small ruminants, via vectors. The initial serological status of the group was unknown. In recently infected population the disease can result in high mortality in offspring. Once infection is established it results in a high morbidity, but a low mortality rate in adults. (HUNTER and KREEGER, 1998). Due to the semi free range type of sandy enclosure it was difficult to notice an increase in mortality of offspring.

Clinical signs by binocular examination of the group of gazelles included clearly visible "hygromas" - enlarged, swollen carpal, tarsal, and phalangeal joints of different sizes which occurred uni or bilaterally. Hygromas were observed in adult (figure 1) of both sexes and in weaned fawns (figure 2). Hygromas of joints described by several authors are pathognomonic for *Brucella sp.* infection in domesticated animals (BALBO *et al.*, 1969, MUSA *et al.*, 1990) and wildlife representative of *Bovidae* family (THORNTON 1975, GRETH *et al.*, 1992, DAVIS, 1990, GARIN-BASTUJI *et al.*, 1990, GRETH *et al.*, 1992, FEROGGIO *et al.*, 1998, GODFROID, 2002, MOLLAH and MCKINNEY, 2002, OSTROWSKI *et al.* 2002, AFRICAN WILDLIFE DISEASES, 2016) and in Camelids (RAMADAN *et al.*, 1998).



Fig. 1: Joints "hygromas" in adult dorcas gazelle  
(© T. Wieckowski)

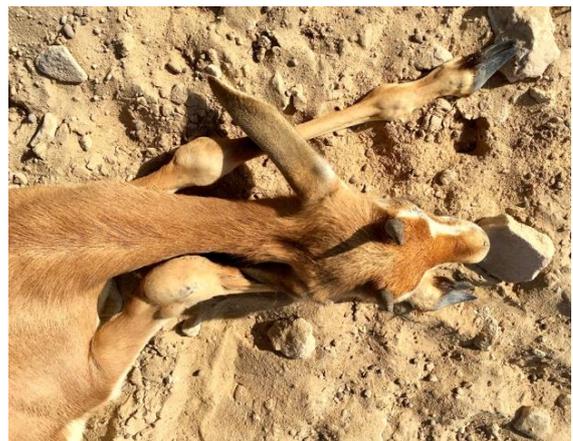


Fig. 2: Joints "hygromas" in dorcas gazelle  
fawn (© T. Wieckowski)

The gazelle population of this particular private enclosure had increased considerably during the past ten years. This population growth was achieved by intensive inbreeding. No external genetic material

had been introduced since last seventeen years. Under favorable environmental conditions, the productivity in this species is quite high. In the absence of an efficient predator coupled with prohibition of hunting, a small population of dorcas gazelles can reach high numbers in a rather short period. Mortality was sporadic, and injuries due to the fight between males was the main cause of fatalities.

It could be also speculated that the birds brown-necked raven (*Corvus rufficollis*), (OSTROWSKI *et al.*, 2002) might have disseminated the infectious agent as a passive carrier to the gazelles, possibly picking up brucella organism by feeding on placentas or aborted fetuses and contaminating feeding and drinking troughs in the gazelle enclosure. The infection could be a result of spill-over of disease from domestic to naïve wild population possibly over long distances.

Firstly there is an urgent need to develop a management programme (BAHARAV, 1983) to check the size of gazelle population and controlled reduction in the number of animals through hunting. This will lead to the stability in the population size. The presence of hygromas in gazelle is evidence of brucellosis in the flock. Therefore, animals suffering, from hygromas represent a potential hazard to both the flock (ALEXANDER *et al.*, 2002) and humans who come into contact with them (BENGIS *et al.*, 2004, PAPPAS *et al.*, 2006) and should be culled. (BAHARAV, 1988) recommended, based on Leslie matrix technique (USHER, 1972) to reduce gazelle population to 26 gazelles per 1 sq.km.

This survey is the first published study on the *Brucella melitensis* infection of a large group of semi free-ranging wild gazelles in the Middle East. Contrary to the previous survey done by (OFFNER *et al.*, 2007) on three large enclosures of semi-free ranging private collection of wildlife bovids in the Emirate of Dubai our investigations indicate, that wild ruminants in a semi free ranging collection may be a source of *Brucella melitensis*.

## Acknowledgements

Author is grateful to the Dubai's Ruling Al Maktoum Family and H.H Sh. Ahmed bin Mohammed in particular, for the constant interest and support of research of the local fauna, leading to better understanding of its health status and natural habitat.

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