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ASSESSING ZOO ANIMAL WELFARE USING ANIMAL-BASED INDICATORS

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Assessing the welfare of zoo animals is necessary to identify problem areas, set priorities for action and monitor progress when improvement strategies are implemented. Animal welfare can be assessed by using a combination of several valid, reliable and feasible indicators. Welfare indicators can be conveniently grouped into resource-based and animal-based indicators. Resource-based indicators have been widely used, particularly when developing best practice guidelines. However, the effect of a given environmental feature on the welfare of animals is not always predictable, as it depends on the temperament of each individual animal as well as on the interaction between environmental features. Consequently, animal-based indicators should be used whenever possible to assess the welfare of animals. The objective of this paper is to review the main animal-based indicators that can be used to assess the welfare of zoo animals, with particular attention to their methodological limitations. Animal based indicators include behavioural changes, physiological parameters, changes in the physical appearance of the animals and veterinary records. Behavioural indicators include both “abnormal” behaviours and changes in the frequency, duration or intensity of normal behaviours. “Abnormal” behaviours are those that are never or very rarely seen in free-ranging animals, such as stereotypies and apathy, for example. Although they are potentially very useful as indicators of poor welfare, caution is needed when using them. For example, stereotypies may be a consequence of past rather than current conditions, and animals differ in their propensity to develop stereotypies in sub-optimal environments. The most easily observed sign of apathy is inactivity, but this has to be assessed considering the species-specific circadian rhythm of activity. Changes in normal behaviours such as feed intake, aggression, maternal behaviour or play, among several others, are also potentially useful, but setting up a range of normal or acceptable frequencies or durations for each of these behaviours is far from easy. Measures of the hypothalamic-pituitary-adrenal axis activity have been widely used to assess stress and welfare, and cortisol in hair is a promising indicator of chronic stress. However, an increase in the synthesis and release of cortisol or corticosterone does not necessarily indicate that the animal has been exposed to an aversive situation. Indicators related to the physical appearance of the animals include body condition, posture, cleanliness of the body surface and facial expression. Finally, data on disease prevalence and life expectancy can be used to assess animal welfare at group level. Some diseases, particularly those that cause pain or other form of suffering and multifactorial ones, are particularly useful to assess welfare. Comparison between life expectancy in zoos and in the wild has been used in several species to assess welfare conditions in captivity; care should be taken, however, when interpreting the results as life expectancy in long-lived species may be a consequence of past conditions. These and other welfare indicators that are well known or are being developed for zoo animals will be discussed in the presentation.

BEHAVIOURAL ANALYSIS OF CAPTIVE TIGERS (*PANTHERA TIGRIS*) UNDER DIFFERENT ZOO MANAGERMENTS

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Summary

Activity budgets of seven captive tigers (*Panthera tigris*), housed in four different zoological gardens (named A, B, C, D), were analysed in order to assess their welfare and to relate it to variables such as enclosure type, management and animal history. An instantaneous focal animal sampling was used to record behaviours with an interval of two minutes. An ethogram of 40 behaviours was adapted from literature. Data were collected by five observers. The behaviours were then categorised into signs of stress, signs of good welfare, and neutral. Statistical analysis were carried out in order to find in which zoo tigers were more prone to exhibit behaviours suggesting good welfare and which feature has major effect on their welfare. Over 195 hours of data were collected and an amount of 5,867 observations were recorded. Tigers seem more prone to express behaviours signs of good welfare in zoo A (OR = 4.11 CI95 % 3.2 - 5.3) and zoo C (OR = 1.83; CI95 % 1.4 - 2.4), using as reference term Zoo D. Presence of a water pool is significantly associated with welfare, even adjusted by other variables such as the time of observation and the presence of a platform (OR = 2.04 CI95 % 1.4 - 3.04). The data analysis suggested that the studied tigers did not display consistent signs of stress (i.e. pacing) and experienced a basic welfare status. An essential feature that helps to enhance good welfare of captive tigers appeared to be a water pool in the enclosure.

Introduction

Tigers (*Panthera tigris*) are frequently housed in zoological gardens due to different factors: they belong to the charismatic megafauna, are easy to breed and are resistant to heat and cold. Unfortunately captive environment differs dramatically from what these animals should experience in their wild environment (SZOKALSKI *et al.*, 2012). Goal of many researches is to find out how to prevent or reduce pacing (BASHAW *et al.*, 2007; MILLER and KUHAR, 2008). However, welfare is not just fulfilling essential needs and preventing the onset of stereotypies, but also encouraging animals to express the most complete behavioural repertoire.

Aim of this multi-zoo study was to assess the welfare of tigers housed in four different zoological gardens situated in Piedmont region, Italy, relating it to variables such as enclosure type, management and animal history. As a consequence, the study aimed also to identify features with a key role in tiger welfare, that could be easy to be implemented in the field and thus that could be recommended to zoos hosting these big cats.

Material and methods

Seven captive tigers, housed in four different zoological gardens (named A, B, C, D), were studied in order to recognise signs of stress or of good welfare related with enclosure type, management and animal history. A and B were traditional zoos, C and D safari parks. All animals, but one, were housed in pairs. Six tigers were born in place, while one was previously owned by a circus.

An instantaneous focal animal sampling was used to record behaviours with an interval of two minutes. An ethogram of 40 behaviours was adapted from literature (BASHAW *et al.*, 2007; MILLER and KUHAR, 2008). Data were collected by five observers. Before beginning the study, the reliability of the observation method has been assessed by Cohen's kappa. Each institution was visited five times from April 2012 to October 2012 and observations were taken when the animals were on exhibition. To process data, activity budgets were constructed. Observations recorded as "out of sight", due to a visual barrier between observers and animals, were excluded from further statistical analysis. The behaviours were then categorised into signs of stress (pacing, aggression, flee, avoid), signs of good welfare (table 1), and neutral (all the others), according to previous literature (KARANTH and SUNQUIST, 2000; MILLER *et al.*, 2013). A multinomial logistic regression model was then carried out in order to find in which zoo tigers were more prone to exhibit behaviours suggesting good welfare. In order to identify which aspects have major effect on tiger welfare, animal peculiarity, daily routine management and enclosure features that could influence welfare status of captive tigers were listed, categorised, and analysed by mixed-effects models for binary and binomial responses. The conditional distribution of the response given the random effects was assumed to be Bernoulli.

Tab. 1: List of behaviours indicative of good welfare.

Behaviours indicative of good welfare
Groom: licking and/or biting any part of own body (no physical indicators of over grooming)
Immersion: entering a water pool with any part of the body other than the mouth
Approach: any non-aggressive social interactions with other animals
Out of sight (animal): animal, on purpose, is in an area which is visually inaccessible to observer
Interaction with environment <ul style="list-style-type: none">- Spray: horizontal ejection of urine against vertical surface;- Mark: claws being drawn or cheeks being rub over any non-animal surface;- Sniff: taking air through the nose in repeated small sniffs;- Flehemen: grimacing facial expression with the tongue out of the mouth while drawing scent over the facial glands;- Listen: lifting up and turning ears in direction of a noise;- Enrichment/environment: any playful behaviour directed towards an object.

Results and discussion

Before beginning the study, the observers reached a within-observer reliability ranging from 0.72 (CI95 %: 0.54 - 0.9) to 1 (CI95 %: 0.82 - 1) and a between-observer reliability of 0.95 (CI95 %: 0.9 - 1). Over 195 h of data were then collected and an amount of 5,867 observations were recorded. In total, 809 observations were excluded from further analysis because the animal was "out of sight". Overall finding of activity budget construction was that tigers spent most of their time sleeping (32.64 %). In addition to that, the most frequently occurring behaviours were resting (27.5 %) and walking (17.3 %). Active time, considered as the time when the tigers were neither sleeping nor resting, was 39.86 %. All tigers were engaged in pacing for a small amount of time (0.43 %). Results of multinomial logistic

regression model are shown in table 2. Tigers seemed more prone to express behaviours indicating good welfare in zoo A (OR = 4.11 CI95 % 3.2 - 5.3) and zoo C (OR = 1.83; CI95 % 1.4 - 2.4). Among enclosure features, the presence of a water pool was significantly associated with good welfare (OR = 2.04 CI95 % 1.4 - 3.04). Animal history, single/pair housing and presence of a log or of an elevated platform in the enclosure did not result significantly associated with good welfare.

In conclusion, the observed tigers did not display consistent signs of stress (i.e. pacing) and experienced a basic welfare status. This is probably the result of the strong efforts that zoos made to reduce abnormal behaviours, operating both on enclosures architecture and animal management. However, the next step that zoo should pursue is to allow their animals to achieve a high level of welfare.

Results provided by the present study state that a water pool has a key role in enhancing welfare status. The implementation of this feature in already existing or newly planned exhibitions is not a big deal for zoos willing to host tigers, in terms of costs and staff commitment. Based on the literature, animal welfare status may also be improved by introducing an enrichment plan, which yet requires more efforts and a good plan by zoo managers (SZOKALSKI *et al.*, 2012). Zoos involved in the study did not perform enrichment systematically and this could be the reason of the missed recognition of this variable as a welfare enhancer.

Tab. 2: Results of multinomial logistic regression model.

Dependent variable	Independent variable	OR	<i>p</i>	95 % CI	
Good welfare	Zoo				
	D (ref.)	1	-	-	-
	C	1.83	0.000	1.41	2.37
	B	1.06	0.691	0.80	1.39
	A	4.11	0.000	3.18	5.30

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HAIR CORTISOL CONCENTRATIONS IN NON-HUMAN PRIMATES AS RELIABLE INDICATOR OF CHRONIC STRESS

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Stress is known to influence hypothalamic-pituitary-adrenal (HPA) axis activity in a variety of species, including fish, deer, birds and humans. Exposure to chronically elevated levels of circulating glucocorticoids results in a host of health problems in animals and humans. This can be assessed by cortisol level measurements in urine, saliva, blood and faeces. However, daily sampling over a long consecutive time period is necessary; salivary and plasma cortisol levels may be confounded by emotional responses to testing procedures (e.g. invasiveness of sample collection) and moreover, saliva, urine and faeces may have a circadian variability that makes the time point of daily sampling crucial.

Recent human studies have identified hair cortisol, measured in timed hair extracts, as a biomarker of chronic stress (reliable indicator of chronic HPA axis activity). Hair grows at approximately 1 cm per month, and retrospectively reflects average stress levels. Therefore, our laboratory, in collaboration with the University of Massachusetts Amherst (USA), developed and validated a reliable method for measuring cortisol in the hair of nonhuman primates (NHP), which reflects cortisol concentrations over several months. We assessed hair cortisol concentrations in rhesus monkeys (*Macaca mulatta*), cynomolgus monkeys (*Macaca fascicularis*) and common marmosets (*Callithrix jacchus*). Age, gender and husbandry and housing conditions were compared. We will present our findings (e.g. site re-location is associated with increased chronic, endogenous glucocorticoid exposure). We will discuss the implications of these findings with respect to studying responses to major life stressors in NHP in laboratory facilities and zoos (e.g. monitoring chronic stress that might be associated with various experimental manipulations, re-location and exposure to zoo visitors).

ASSESSMENT OF FAECAL GLUCOCORTICOID METABOLITES IN HEALTHY AND ALOPECIC ANDEAN BEARS (*TREMARCTOS ORNATUS*) IN GERMANY

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The Alopecia Syndrome of Andean bears (*Tremarctos ornatus*) has become a serious issue for zoos in the last years. Chronic hair loss and pruritus as the main symptoms are an animal welfare problem and only few symptomatic treatments are known yet. Several case reports assumed a link between declining skin condition and stressful events. The aim of that study was to analyse the impact of adrenal activity in the disease and to get first information about risk factors that might support a deterioration of an existing skin disorder.

Faecal samples of twelve bears (four males, eight females) in five German zoos were collected once to twice weekly throughout one year. All males and three female bears had intact skin conditions, while five females were suffering from alopecia in different stages. Samples were freeze-dried and pulverised prior to extraction. Steroids were extracted using methanol and analysed with a radioimmunoassay for cortisol. Validation procedures showed parallelism to the standard curve, a recovery rate of 93 %, and intra- and interassay coefficients of 2.6 % and 9.1 %, respectively. For every individual a mean of the hormone concentration of all samples was calculated. All data were analysed for their influence on factors such as age, sex, coat condition, and housing conditions. For correlation of age a Spearman-Rank-test was used. Comparisons were carried out using Mann-Whitney-U-test.

A correlation of age and glucocorticoid values ($P = 0.721$) and an influence of sex ($P = 0.386$) could not be found. Also a statistic significant difference in glucocorticoids of healthy and affected bears was not detectable ($P = 0.786$). The individual mean values ranged in affected females from 156 ng/g to 634 ng/g, whereas in healthy females they ranged from 230 ng/g to 431 ng/g. Disregarding alopecia we found significant higher values for animal in enclosures $< 150 \text{ m}^2$ compared to larger enclosures ($P = 0.008$) and in male-female-pairs compared to single-sex groups ($P = 0.03$).

This study is a first approach to the endocrine influences in the Andean bear Alopecia Syndrome. We could not find significant different values in the mean hormone concentrations of alopecic bears. Their wider range is possibly due to small sample size. Enclosure size and group structure were influential to adrenal activity in our study group. Even though sample size was small, these results underline existing husbandry guidelines and should be considered in husbandry and management of already affected animals.

VALIDATION OF AN ENZYME IMMUNOASSAY FOR THE MEASUREMENT OF SHED SKIN CORTICOSTERONE IN KOMODO DRAGONS (*VARANUS KOMODOENSIS*)

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Glucocorticoid (GC) levels are often measured to quantify stress. Integumentary structures of keratin, such as reptile shed skin, have recently been reported to accumulate GCs. The aim of the present study was to evaluate if a commercial enzyme immunoassay (EIA) and an optimised corticosterone extraction method were appropriate for the quantification of corticosterone levels in Komodo dragon (*Varanus komodoensis*) shed skin. Opportunistic samples from two males and four females were collected non-invasively for a month at Barcelona Zoo. Skin derivatives were classified according to the anatomical region they came from. After hormone extraction, corticosterone quantification and validation tests were performed using a commercial Enzyme-linked Immunosorbent Assay (ELISA) kit (Neogen[®] Corporation, Lexington, KY, USA). Extracts from 22 samples were used to constitute control pools. The precision or intra-assay CV was 5.96 %, confirming the repeatability of results. The serial dilutions of the pooled extract, performed in the dilution test, displayed a good correlation coefficient ($R^2 = 99.41$ %) between expected and obtained values. The slope of the corticosterone standard curve was parallel to the slope of displacement curve based on the serial dilution of the pooled extract ($\text{slope}_{\text{standard}} = 0.61$; $\text{slope}_{\text{pool}} = 0.67$). The mean rate of hormone recovery obtained in the spike recovery test was 110.33 ± 22.48 %, suggesting that other components did not interfere significantly with the hormone estimation. The sensitivity of the test was 0.033 ng/ml. Results obtained show for the first time that, following the hormone extraction protocol, corticosterone deposited in Komodo dragon shed skin can be detected and measured by this EIA. Physiological and biological validations should however be performed before applying it in this species. Although demographic, genetic and behavioural studies of wild and captive Komodo dragons have been conducted, GC levels have never been measured previously in this species. Our method may thus have important applications for their conservation and the effective management of the species in captivity.

PRACTICAL ZONOSIS MANAGEMENT FOR ZOOS

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Zoo veterinarians have an increasing responsibility for their organisation's compliance with health and safety and public health legislation, especially to ensure the protection of employees, visitors and animals from zoonotic diseases.

Inadequate management of a zoonotic disease, either through overreaction or failure to respond adequately, may have serious consequences. It can result in unnecessary human and animal illness, compromised public health and, through misdirected media attention, can lead to public concern and impact on the reputation of the institution and the responsible veterinarian. To prevent this, and to handle these situations consistently and professionally, it is recommended to have a systematic management plan in place (DEFRA, 2012; DONALD, 2012). However, development of a comprehensive plan requires a great deal of thought and work as well as consultation with multiple stake holders such as Human Resources, Health and Safety, Public Health, curators, animal staff and others. This results in a complex and lengthy process which can be difficult for an institution to undertake.

Through a series of examples, we present a practical and comprehensive zoonosis management process that has been developed for Chester Zoo over several years. This is a modular system, covering different areas in zoonosis prevention planning (visitor protection, staff protection, and animal protection), through risk assessments, biosecurity protocols and safe working procedures. It also covers zoonosis contingency and response protocols for the management of a zoonotic disease detected in the zoo. These include lines of communication and responsibility, disease fact sheets, and training.

The whole system or the individual modules can be easily adopted or used as a template by zoological institutions to develop their own plans.

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BRONCHOALVEOLAR LAVAGE FOR TB DIAGNOSIS IN RHINOCEROS – IMPACT OF TB DIAGNOSIS ON ANIMAL WELFARE

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Pathogenic mycobacteria in human, livestock and endemic wildlife species bear a great threat to endangered species in zoological institutions. Upon Tb diagnosis in a zoo collection, the risk of zoonosis for zoo staff and visitors or infection risk for other animals is of high concern. By law the zoonotic risk mandates further Tb testing of potentially infected animals or of the whole animal collection. Yet, immunological methods for Tb diagnosis established in human and livestock (skin test, serological test, γ -interferon) are not validated in wildlife species. Specifically, false positive results from non-validated serum tests might have fatal consequences as animals might become subject to euthanasia. In this context it is surprising that bacterial culture from bronchoalveolar lavage, the gold standard for Tb diagnosis in human, seems yet greatly underutilised in wildlife species to further determine the presence of mycobacteria. In this study we investigated the feasibility of broncho-alveolar and oesophageal lavage in seven white rhinoceroses (*Ceratotherium simum simum*) from two zoos as a complementary method for Tb diagnosis for non-validated immunological tests. Tb had been diagnosed *post mortem* in other species. Positive serological tests (DPP) in five rhinoceroses suggested a Tb infection. Bronchoalveolar and oesophageal fluids were collected from all animals and tested for the presence of *Mycobacterium tuberculosis* complex (MTC) bacteria by PCR, bacterial culture and real time PCR. Samples from all animals were PCR negative directly after sample collection. After 12 weeks of bacterial culture weak evidence on the presence of mycobacteria was found in oesophageal samples from two animals. In one animal the positive culture was associated the presence of non-tuberculous mycobacteria but not of members of the MTC. In the other animal *Mycobacterium tuberculosis* was finally identified in the oesophageal sample by real-time PCR and microarray spoligotyping. Yet, all results negative and positive require re-investigation. Negative results may just indicate that animals were not shedding at the time of sampling. For the animal tested positive the result must be confirmed prior further conclusions. It is concluded that in absence of validated immunological tests for Tb in wildlife, bacterial culture, PCR and real time PCR from samples obtained by bronchoalveolar lavage, represent a feasible and complementary method for Tb diagnosis in rhinoceros providing additional data that allow evidence-based management decisions.

MYCOBACTERIUM TUBERCULOSIS INFECTION IN TWO AFRICAN ELEPHANTS (*LOXODONTA AFRICANA*)

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Tuberculosis, caused by *Mycobacterium tuberculosis*, is an important cause of morbidity in captive elephants worldwide. Primarily infected humans present a threat to elephants; subsequently infected elephants pose a threat to their keepers and the public. Particularly since overt clinical illness typically only develops in advanced disease. Compared to the range of tools available for diagnosis of tuberculosis in humans, the range of validated tools for detection of the disease in elephants is limited. Both intradermal skin testing and trunk-washes are hampered by poor sensitivity. Valuable diagnostic tools for this species are sero-diagnostic tests (the ElephantTB STAT-PAK, Dual Path Platform [DPP] VetTB (ChemBio Diagnostic Systems Inc., Medford, NY, USA). The interferon gamma (IFN- γ) assay suffers from inconsistencies and is not yet validated. Two adult female African elephants (*Loxodonta africana*) were re-allocated from a Spanish zoo to a zoo in the Netherlands in 2013. Prior to transportation the animals were tested for *M. bovis* / *tuberculosis* infection with ELISA-tests by The Central Veterinary Institute (CVI), Lelystad, The Netherlands, and were considered negative. Eighteen months after arrival, one of these elephants presented with vaginal discharge and weight loss. The animal was anaesthetised and a skin test performed in the outer ear margin with bovine purified protein derivate (PPD-B) tuberculin. The result was inconclusive. Blood was collected for serology. ELISA-test and IFN- γ assay were performed several times and both tests became positive over time. Due to the risk of zoonotic transmission, the animal was euthanised and necropsy was performed. All lymph nodes were swollen and showed mineralisation on cut surface. Purulent-haemorrhagic material was present in the trachea; lungs and kidneys contained multifocal granulomatous lesions of various sizes; the uterus was filled with purulent material. Ziehl-Neelsen staining (ZN) of cytology from all but the renal lesions contained abundant acid fast bacteria. Histologically, granulomatous lesions with mineralisation, focal necrosis and multinucleated giant cells were present in the lymphnodes, lung, kidney and uterus. In addition, PCR and culture of these tissues were positive for *M. tuberculosis*. Both the contact female and another male elephant in the Netherlands subsequently tested positive. The second female was also euthanised and at necropsy enlargement, necrosis and mineralisation of the parotid, prescapular and bronchial lymph nodes was observed as well as a single acid fast bacterium in the ZN stain of the parotid lymph node. On histological examination, the lymph nodes were reactive, with scattered multinucleated giant cells and minimal necrosis. Culture was positive for *M. tuberculosis* after four months. All keepers and necropsy attendants underwent Mantoux tests and of the four positives, two were also positive in the IFN- γ assay and are now being treated with isoniazid (300 mg) and rifampicin (600 mg), q 24 h (Rifanah 300/150, Eureco-Pharma, Ridderkerk, The Netherlands). At this point the male elephant as well as two remaining female elephants are being monitored with the available ELISA's and IFN- γ tests. Trunk washes with direct culturing of mycobacteria are planned and the elephants are currently being trained for these procedures.

VETERINARY SERVICES AND RESEARCH SUPERVISION FOR NON-GOVERNMENTAL WILDLIFE WELFARE ORGANISATIONS

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The veterinary team at the IZW functions as advisor for many wildlife projects worldwide. During the last decade the demand of veterinary support and research supervision for non-governmental organisations (NGO's) increased a lot. Due to the increased wealth and education in (parts of) the human population, ethical awareness of animal welfare increased likewise. Wildlife welfare projects deal, amongst many others, with cases of: a) improvement of husbandry, enrichment and diets in wildlife enclosures, b) illegal wildlife trading, trafficking and abuse, c) illegal use and poor husbandry, d) the improvements for these animals' situation when rehabilitated at sanctuaries. Wildlife welfare projects often have overlapping interests with wildlife crime projects as both often go hand in hand. Wildlife crime became the fourth largest transnational crime worldwide, worth at least 17 billion US Dollar (for the year 2014). Cross border and inter-agency collaborations are necessary to conquer these problematic situations, and veterinarians and scientists can make great contributions to solutions and improvements. The intensive co-operation between the IZW and the NGO 'Four Paws International' in the sector of illegally kept large carnivores in Europe and South-East Asia deals with: dancing bears, restaurant bears, bile-farming bears, baiting bears or carnivores in illegal husbandry. A large amount of veterinary case load was collected over the last years, which indicated the former, improper keeping of such individuals. Pathologies, especially in the musculoskeletal system are seen in many of the rehabilitated animals. Arthrosis, spondylarthrosis and discus prolapses are a common consequence when keeping animals on inflexible, concrete floor (movement shock absorption within the joints and not the soil) in combination with a severe lack of movement and hence insufficient support of the joints by strong skeletal muscles. Computer tomography nicely shows the pathologies in vivo. As a proposed solution we started a study on long-acting painkillers (Mavacoxib, Trocoxil, Zoetis, Zurich, Switzerland) and geriatric medications (Propentofyllin, Karsivan, Intervet, Unterschleißheim, Germany) in comparison to standard pain protocols with daily medication (meloxicam, Metacam, Boehringer, Ingelheim, Germany) for brown bears ($n = 9$). Dental pathologies are often diagnosed in animals with incorrect diets (rich in carbohydrates; garbage-food) and the abnormal behaviour such as chewing on metal poles due to boredom within an impoverished enclosure. These cases are always referred to a wildlife dentist. Within these projects we have developed and improved a new anaesthetic protocol (using four components 2.5 mg/kg Ketamin (10 % Essex, Bad Arolsen, Germany), Medetomidine (4 %), Midazolam (5 %), Butorphanol (5 %), all from Dr. S. Quandt, Bryanston, RSA) for brown bears ($n > 60$), tigers ($n = 28$) and lions ($n = 24$) which has a medium-long duration with a quick recovery and good vital parameters, but is also easy to prolong in the field, if necessary. Furthermore, we started an ultrasonography study on Asiatic black bears, rescued from bile-farming stations, which shows the pathognomonic lesions, typical for bile extractions. Within a stress-research project we could show the differences of adrenal gland morphology (ratio between cortex and medulla) in 3D ultrasonography. Measurements in hair and serum cortisol are used comparatively and the measurements are repeated one year after rehabilitation in a sanctuary to show the effect on the physiology of the adrenal glands.

CONSTRUCTION OF AN INTEGRATED WELFARE ASSESSMENT SYSTEM (MACWEL) FOR MACAQUES (*MACACA SPP.*) IN HUMAN HUSBANDRY

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Summary

The paper describes the construction procedure of an assessment protocol for Macaques (*Macaca* spp.). The authors have chosen an integrative approach, currently frequently used for the construction of such protocols with a clear preference for animal-based parameters to assess the welfare states of these non-human primate species in human husbandry. In collaboration with researchers of this field valid, reliable and feasible indicators were composed to a holistic assessment protocol; agreement was reached with a Delphi method. The four principles from Welfare Quality were used as definition for Animal Welfare, whereas the 12 corresponding criteria were enriched with additional attributes: Comfortable Lighting, Absence of Noise, Ease of movement and natural habitat. The authors will further proceed with using more experts in a Delphi method for the translation procedure to aggregated levels to be able to arrive at an overall score for each assessed facility.

Introduction

Animal welfare is known to concern not only physical health and needs (BROOM, 1996), but also the extent to which the animals' natural lifestyle is satisfied, (ROLLIN, 1993) and the state of the animal's mind. Animal welfare is strongly influenced by the mental state experienced by animals: the absence of strong negative feelings ("suffering") and the presence of positive feelings ("pleasure"). In any assessment of welfare, it is these states that should be evaluated (DUNCAN, 1996). One suggested approach used the opinions of researchers, including veterinarians, to devise a set of indicators for assessment of the welfare, arising from animal-based parameters such as behaviour and clinical examination, but as well from resources and management if necessary (SØRENSEN and FRASER, 2010). There is a large body of ready-made assessment systems and data on the welfare of farm and laboratory animals (BLOKHUIS, 2008), but rarely there are assessment systems and data available on wild animals living in human husbandry (HILL and BROOM, 2009). The construction and application of such assessment protocols requires knowledge of animal welfare sciences, animal health and production, and species-typical behaviour, physiology and anatomy (BRACKE, 1999; BRACKE *et al.*, 1999). Knowledge of the latter may come from studies of the species' lifestyle in the wild (CLUBB and MASON, 2007). The aim of our study was the construction of an applicable assessment system (MacWel) on the welfare of a wild species, frequent in human husbandry for different purposes, the macaques (*Macaca* spp.).

Material and methods

Several animal welfare assessment systems available for farm animals, and therefore on group level, have been studied (BLOKHUIS *et al.*, 2003) and the 'Four principles' of animal welfare 'Good feeding', 'Good housing', 'Good health' and 'Appropriate behaviour' (BOTREAU *et al.*, 2009) and the corresponding twelve criteria have been chosen as basic structure to develop the assessment protocol MacWel (figure 1). For each criterion the literature available in PubMed, CAB, and Science Direct searched with the keywords: 'Animal Welfare/ Assessment' and Macaca' was reviewed on potential single measures that could represent the state of the animal regarding these aspects (WELFARE QUALITY[®], 2009; WOLFENSOHN and HONESS, 2005). A requirement for the single measures was further that they were primarily animal-based (WHAY *et al.*, 2003a) and that the parameter goodness, in principle validity, reliability and feasibility, was satisfying (KNIERIM and WINCKLER, 2009). Moreover the parameter had to assess animals at group level as the goal was to evaluate the welfare state of the animals for the whole facility. A list of potential indicators was discussed with two veterinarians for primates and animal welfare/ assessment regarding their applicability to macaques and amended with regard to their comments with the help of the Delphi method to achieve agreement among experts (WHAY *et al.*, 2003b). The natural situation of the animals was used as positive reference if applicable, whereas for the criteria arising from 'Good health' the undisturbed integrity of the animal was the representing the ultimate positive range. For the principles 'Good human animal relationship' and 'Positive emotional state' the magnitude of the positive end was the absence of negative states such as fear, stress or pain. According to the expert options the final list of single parameters (or output-measures) was constructed and the weighting of the outcome-measures with regard to the welfare state, ranging from poor to good, was translated to points between 0 and 100 for the criterion and principle levels.

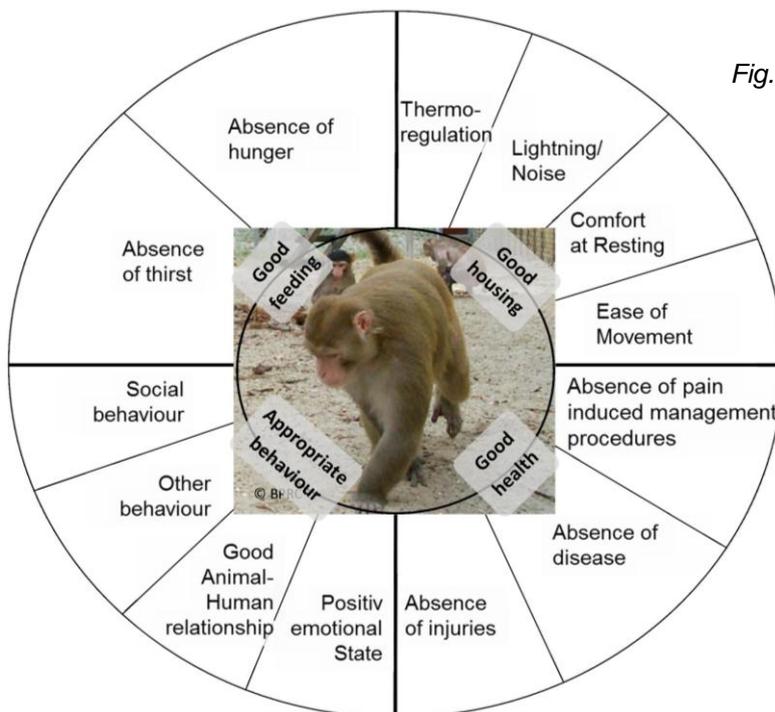


Fig. 1: Principle structure of the MacWel assessment protocol, underlying four principles and twelve corresponding criteria of animal welfare. (Photo: © BPRC)

Results and discussion

The final protocol consists of a brief background description of the 32 single measures, how they shall be evaluated and in which format or unit the outcome-measure shall be recorded. With regard to the current holistic approach of animal welfare sciences a combination of sources are compiled: indicators are preferable assessed directly on the animals, but as well through interviews with the animal care takers or facility manager, and due to inspection of resources and management on-site. A shortlist of the criteria and linked single measures can be seen in table 1.

The whole description of the protocol would be too comprehensive to be described here in detail but some examples shall be given.

1) In criteria 3 'Comfort of resting and natural habitat' the single measure is constructed of resources and an animal-based component and is representing the 'naturalness approach' of animal welfare assessment (ROLLIN, 1993): 'The housing facilities shall provide solid floor and additionally elevated platform or the-like structures, big enough for at least one animal to lie down at one place; separate resting places, at least one per animal shall be present, and have sight barriers and/or coverage. On the floor substrate height shall be ≥ 5 cm to enable foraging. The corresponding outcome measures recorded are: 'percentage of animals provided with adequate resting areas' and 'percentage of animals provided with adequate floorage'.

2) Another example comprises animal-based parameter arising from clinical examination, representing the physiological approach of animal welfare (BROOM, 1996), within the principle 'Good Health' in the criterion 6 'Absence of injuries': 'All animals in a group are checked visually for hairless patches and extensive hair thinning, any kind of fresh or healed wounds and swellings independent from the cause (hair-pulling, fights, disease). Missing parts of the body such as fingers, toes and parts of the tail are regarded as lesions. In groups > 36 animals a calculated sample size will be investigated, the composition will be adapted according to the group structure. The outcome measures recorded are: 'percentage of animals with hairless patches and the average number', 'percentage of animals with lesions and the average number', 'percentage of animals with swellings and the average number'.

3) The 'mental state' approach of animal welfare (DUNCAN, 1996) is given, included in the principle 'Appropriate Behaviour' and the criterion 12 'mental and emotional positive state': The 'Qualitative behaviour assessment (QBA)' (WEMELSFELDER and LAWRENCE, 2001) shall be performed by QBA-educated/trained animal care takers on a regular base on group level. The result of the QBA shall be positive at any time of assessments. The recorded outcome-measure in this case is 'percentage agreement of the average QBA by the animal care takers with the QBA performed by the external assessor'.

According to the researchers and veterinarians opinions results from the single measures will be translated to a range of 0 - 100 points, representing an animal welfare state from poor to good (BROOM *et al.*, 1996). We will use again the Delphi method to achieve agreement among those experts (WHAY *et al.*, 2003b). This strategy has already been proved to be successful in larger projects with more species involved (BOTREAU *et al.*, 2009). Scores on criteria level will be formed out of the average achieved in the corresponding single measures and the same procedure will be performed on the level of principle scores. The final overall score for the facility will result from the average scores of all four principle scores.

Tab. 1: Principles, criteria and single measures included in the MacWel protocol for assessing animal welfare in Macaques on group level in human husbandry.

Principles	Criteria	Measures
Good Feeding	1/ Absence of prolonged hunger	percentage of animals with exceeding BCS (< or > 3)
		percentage of animals with exceeding weight
	2/ Absence of prolonged thirst	amount of animals/drinker
		percentage of clean water points
Good Housing	3/ Comfort of resting & appropriate floorage	percentage of animals provided with adequate resting areas
		percentage of animals provided with adequate floorage
	4a/ Thermal comfort	percentage of animals provided with ambient thermal conditions
		percentage of animals displaying thermo-regulative behaviour
	4b/ Comfortable Lighting	percentage of animals provided with suitable light conditions
	4c/ Absence of Noise	percentage of animals not able to avoid excessive noise levels
percentage of animals provided with enough space		
Good Health	5/ Ease of movement and natural habitat	percentage of animals provided with a permanent access to an outdoor loafing area
		percentage of animals having permanent access to a natural (imitated) environment
	6/ Absence of injuries	percentage of animals with hairless patches & the average number
		percentage of animals with lesions and the average number
percentage of animals with swellings and the average number		
7/ Absence of disease	prevalence of animals with diarrhoea	
	prevalence of animals with diabetes	
	prevalence of animals having clinical signs of severe illness	
	mortality of animals within one year	
8/ Absence of painful procedures	percentage of animals with mutilations	
	percentage of animals identified visually by the animal care takers	
9/ Expression of social behaviour	percentage of animals living in a social structure adequate to their age and sex	
	agonistic behaviours per animal/hour	
	socio – positive behaviours per animal/hour	
Appropriate Behaviour	10/ Expression of other behaviours	percentage of groups displaying normal time budgets and normal ethograms
		percentage of animals displaying stereotypic behaviour at any time
	percentage of groups with environmental enrichment present and animals engaged with it	
11/ Good human-animal relationship	percentage of animals showing fear reactions to humans	
	percentage of animals of the facility presented to a squeeze compartment	
12/ Mental and emotional positive state	percentage agreement of the QBA by the animal care takers with the QBA performed by the external assessor	
	percentage of animals suffering from prolonged pain in the facility	

Future perspective

The protocol will be tested in spring 2015 in one big research facility keeping several hundred animals and further institutions having macaques in husbandry were approached for collaboration. From our pilot study we concluded that it is feasible to assess the animal welfare with the present version of the MacWel protocol. The analyses of the initial and further assessment results will be used to further refine and amend the protocol as well as the scoring- and aggregation system for an overall classification of the facilities.

Acknowledgements

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NAKED MOLE-RAT HUSBANDRY, DIAGNOSTICS AND WHAT WE CAN LEARN FROM THE WILD

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The naked mole-rat (*Heterocephalus glaber*) is a unique rodent species famous for its eusocial lifestyle. In underground-dwelling colonies of up to 300 individuals, generally only one female reproduces together with one to several males. Naked mole-rats possess several traits that are highly unusual among mammals. Most notably, their extreme longevity and low susceptibility to diseases, including cancer-resistance, currently attract extensive scientific and public attention, rendering them increasingly popular for laboratory husbandry and zoo-displays.

To optimise husbandry and animal welfare, it is essential to gain better insight into the needs and requirements of a species by understanding the relevant factors in its original habitat. To this end, we assessed different environmental and health-related parameters of naked mole-rats in the wild, acquired during two field-excursions to Ethiopia in 2013 and 2014 (290 individuals captured from nine different colonies). Comparison was made to naked mole-rats maintained in artificial plexiglass tunnels and chambers at the IZW since 2008, which continuously and successfully breed and display very low mortality (211 individuals kept in 11 colonies).

(i) To assess basic ambient factors in the wild, a custom-built endoscope of 3.5 m length was inserted into the tunnel systems of naked mole-rat burrows. Inside temperature and humidity varied between 23.4 - 29.6°C and 90.1 - 92.8 %, slightly different to conditions in our captive colonies of 27.2 - 32.2°C and 68.8 - 98.2 %, respectively. As naked mole-rats are functionally poikilothermic, these values corresponded well to body temperatures of 31.6 ± 2.0 ($n = 112$) in the wild and 32.0 ± 1.1 °C ($n = 53$) in captivity. (ii) Further, gas composition (i.e. O₂, CO₂, NH₃) of inhabited burrow systems in the wild was compared to that of captive colonies. Higher values were found under captive conditions especially for NH₃ (0.0 vs. up to 0.36 ppm) but also for CO₂ (0.10 – 0.37 vs. 0.08 – 1.37 vol%) and lower values for O₂ (20.1 – 20.9 vs. 19.1 – 20.9 vol%), indicating burrow ventilation in the wild to be more efficient than previously assumed. (iii) As a general measure for health, blood parameters of wild and captive individuals were compared: haematocrit averaged 49.1 ± 4.4 % ($n = 18$) and 47.0 ± 6.3 % ($n = 10$), respectively; for both, white blood cell counts from blood smears were dominated by lymphocytes closely followed by neutrophils. Also, to our knowledge for the first time, we systematically established blood chemistry normal values of healthy captive naked mole-rats. (iv) Using high-resolution ultrasound and *post mortem* histology, we detected conditions indicative of challenges faced in the wild that can be controlled for in captivity (e.g. putatively parasitic skin lesions and dehydration), as well as husbandry-related factors contributing to problems in captivity: the occurrence of several pathological findings potentially associated with factors such as low humidity, high NH₃, suboptimal temperatures or limited space (e. g. nephrocalcinosis, respiratory infections, fighting injuries) will be discussed.

To sum up, we describe several differences between wild and captive environmental variables and health parameters which are helpful to optimise husbandry and animal welfare in captivity.

PHARMACOKINETIC ALCHEMY: INTERPRETATION, LOGISTICS AND ETHICS IN EXOTIC ANIMAL MEDICINE

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Summary

Frequently, zoo and wildlife veterinarians are challenged to identify effective doses of medications for their patients. Drug regimens developed by clinical experience or interspecies scaling often have not been assessed rigorously in non-domestic species. These approaches may lead to erroneous treatments, even when close taxonomic models are used, due to species differences in anatomy or physiology. Identification of such complications can be found by application of pharmacokinetics, or numeric modeling of the physiologic processing of a drug by the animal. Revisiting the principles of this discipline is important for interpretation of published studies. Understanding the logistics of such a project permits effective application of the literature to the clinical patient. Opportunities for pharmacologic investigation can be identified by knowledge of this background. Additionally, pharmacokinetic studies raise ethical considerations, not only for the researchers involved, but also for clinicians using the results. Enhanced familiarity with the sum of these pharmacologic matters will encourage the clinician to apply them in their daily practice.

Introduction

In exotic animal medicine, identification of effective and safe dosing regimens is a challenge (HUNTER and ISAZA, 2008; TOUTAIN *et al.*, 2010). Few medications are approved specifically for non-domestic species (PAGE and PAPICH, 1997). Even with these drugs, actual clinical trials are rare and certainly are unable to encompass the substantial taxon variability for which zoo and wildlife veterinarians are responsible. Clinical experience may provide a starting point for a treatment in a novel species. Calculations through allometric scaling have provided scientific adjustment to some anecdotal administrations. However, even with taxon approximate domestic or comparable exotic animal models, translation of these doses beyond the originally treated species introduces not only risk of potential toxicity (SANCHEZ-MIGALLON GUZMAN *et al.*, 2010; HYATT *et al.*, 2014) but also outright inefficacy (HUNTER and ISAZA, 2008; TOUTAIN *et al.*, 2010).

Although a successful prescription must encompass the drug's effects (pharmacodynamics), it actually is the animal's effect on the drug (pharmacokinetics) which introduces more interspecies variability (TOUTAIN *et al.*, 2010). Some of these concerns can be interpreted from knowledge of the drug's absorption, distribution, metabolism, and elimination, and its interactions by pH within the body compartments – only, however, if these are assessed within each target species (PAGE and PAPICH, 1997; RIVIERE, 1997; TOUTAIN *et al.*, 2010). For exotic animals, the mathematical modelling from structured analysis of plasma drug concentrations against time is the more straightforward of the pharmacologic assessments available (KUKANICH, 2011). This review encompasses reminders of terminology and formulae which are very straightforward, yet critical for application of such studies. Furthermore, examination of the principal components of a successful pharmacokinetic study through current literature is presented. Lastly, ethics of obtaining and applying this information is considered. It

is anticipated that critical thinking for this discipline will encourage clinicians to incorporate these analyses more effectively into their routine patient care.

Interpretation - terminology and formulae

Pharmacokinetic modeling begins by tissue drug concentrations, usually from serum or plasma, graphed on the y-axis semi-logarithmically (base 10) against time of the sample collections on the x-axis. From this simple representation, even by pencil and calculator, one can identify the major components of a drug's manipulation within a given animal system. The shape of this curve varies clearly by route of drug administration, but a model should be assigned beyond the pattern description (RIVIERE, 1997) unless non-compartmental analysis is utilised. Steeper curves from intravascular dosing can be distinguished from the more gradual slopes of extravascular administration or the bell-shaped curves of oral or depot formulations. Their space encompassed is described numerically as the area under the curve (AUC). This measure is one of cumulative drug exposure and accommodates both plasma concentrations and the duration these concentrations are present (KUKANICH, 2011).

A best-fit line drawn along the steepest portion of the curve must incorporate a minimum of three data points and assesses distribution (line A). A subsequent, similar, best-fit line to the flatter portion of the curve will represent elimination (line B). These lines will be associated with y-intercept concentrations (A and B) and slopes (α and β). An estimated AUC can be calculated by summing the quotients of intercept and slopes, usually represented as $(A/\alpha) + (B/\beta)$ (RIVIERE, 1997). From these initial evaluations, one dependent and two independent parameters can be produced as the primary focus of practical pharmacokinetic interpretation.

Volume of distribution (VD)

This apparent volume exists nowhere within the body but may be compared against total body water (0.6 - 0.7 L/kg) and as such is affected markedly by species, signalment, and health status. Volume of distribution (VD) is considered the independent measure of the fluid required to reach the dilution of the drug measured in the plasma. While specific location of the distribution cannot be assigned, this value relates to the penetration of the drug to body tissues when high (> 1 L/kg) and may include both intended and unintended points of distribution (GOUDAH and HASABELNABY, 2013). Conversely, when it is low (< 0.3 L/kg), minimal distribution from circulation is indicated such as that produced by protein binding restricted movement of free drug (RIVIERE, 1983).

This value is calculated directly from the plasma concentration-time curve. However, it may be considered from several different vantages during the pharmacokinetic analysis, therefore may be confusing for the casual reader. It is considered most robust when evaluated either at steady-state (VD_{ss}) or by area (VD_{AREA}) when most of the pharmacokinetic process is incorporated (RIVIERE, 1997). Specifically, it may be calculated by one of the following formulae (RIVIERE, 1997; KUKANICH, 2011):

$$(a) VD_{AREA} = DOSE/AUC \times \lambda$$

$$(b) VD_c = D_{OSE}/A+B$$

Clearance (CI)

As the second independent parameter, total body clearance (CI) summates all routes of elimination that remove the administered drug from a given volume of plasma expressed against time (RIVIERE, 1997) and, often, in context of animal weight. Although many of these routes relate to renal elimination, especially for drug molecules of smaller molecular weight, hepatic extraction is key for the larger molecules; in neither situation are these processes necessarily predictable by close taxonomic association (TOUTAIN *et al.*, 2010). Clearance may be calculated either from VD or directly from the curve as follows (RIVIERE, 1997):

$$(a) CI = VD \times \lambda$$

$$(b) CI = D_{OSE}/AUC$$

Elimination half-life ($t_{1/2}$)

Although on first consideration it seems independent, the parameter of half-life ($t_{1/2}$) actually is created from the independent parameter of slope of the elimination line (β), which also may be designated as

λ , and equals 0.693 divided by the slope of the terminal elimination line (RIVIERE, 1997). The definition of $t_{1/2}$ often is misunderstood and is the time required to eliminate 50 % of the plasma concentration of the drug remaining within the body (KUKANICH, 2011). While this time interval is constant, the absolute quantity of drug excreted changes with each half-life (TOUTAIN and BOUSQUET-MÉLOU, 2004, KUKANICH, 2011). Withdrawal times and this parameter are associated frequently as the number of half-lives elapsed can be assigned to the overall elimination of an administered drug dose, specifically three $t_{1/2}$ with 88 %, five $t_{1/2}$ with 97 %, and seven $t_{1/2}$ with 99 % (KUKANICH, 2011). Additional practicality of this parameter results from its application to calculate dosing interval to maintain appropriate drug accumulation and maintenance of systemic plasma concentrations at steady-state. When reported as an average, $t_{1/2}$ should be determined as a harmonic mean with pseudo-standard deviation (TOUTAIN and BOUSQUET-MÉLOU, 2004). This approach stems from its origin as a calculation from the independent slope rather than it being a direct measurement. If one chose instead to create a typical mean and standard deviation from the slope and then calculate $t_{1/2}$, it would be found most coincident with the harmonic mean rather than the inaccurate representation which results from averaging a calculated rate.

Elimination half-life must be used with caution when an extended absorption phase is present in the pharmacokinetic processing of a given drug. Ignoring this potential, especially when depot or long-acting formulations are administered, and not performing an intravenous elimination study is a frequent error in $t_{1/2}$ interpretations. In these situations, the terminal slope may change locations with the absorption curve, as it is no longer the rate limiting step. When this “flip-flop” phenomenon occurs, analysis of the terminal slope will not provide accurate measure of $t_{1/2}$ (TOUTAIN and BOUSQUET-MÉLOU, 2004).

Bioavailability

An additional consideration in curve comparison is that of bioavailability (F) expressed as a percentage. For calculation, this parameter must have an intravenous study to serve as the completely absorbed denominator. The numerator is an extravascular route and when the administered doses are not equivalent, an adjustment must be made (RIVIERE, 1997):

$$F = \frac{(\text{AUC}_{\text{non-iv route}} \times \text{D}_{\text{OSE iv}})}{(\text{AUC}_{\text{iv}} \times \text{D}_{\text{OSE non-iv route}})} \times 100$$

Interpretation - “The Healthy Hallows”

Although VD, Cl, and $t_{1/2}$ are physiologic representations and influenced by such processes as circulation, glomerular filtration, and metabolism, only select medications actually can be adjusted allometrically through pharmacokinetic parameters (SMITH, 1991; RIVIERE *et al.*, 1997; KUKANICH, 2011). Limitations to interspecies scaling are introduced by marked variability in isoenzymes or saturation elimination routes for hepatic metabolism; interspecies differences in protein binding; or variability in urine pH which profoundly may alter tubular resorption (SMITH, 1991; TOUTAIN *et al.*, 2010). When a drug can be addressed metabolically, it generally is an antibiotic as this category is dependent more on body fluid concentration relative to a bacteria rather than the patient (TOUTAIN *et al.*, 2010). However, even this statement is not concrete as many exceptions exist, including an especially notorious example from the front-line of exotic animal medicine, the quinolones (PREZANT *et al.*, 1994; RAPHAEL *et al.*, 1994, GAMBLE *et al.*, 1997; HAWKINS *et al.*, 2011; HARITOVA *et al.*, 2013).

Despite these interspecies constraints, clinically, these three parameters are related in a conjoined formula which can address individual animal issues and serve as comparison to the pharmacokinetic literature. Dependent on both drug distribution (VD) and rate of elimination (Cl), half-life is related by the following relationship: $t_{1/2} = (0.693 \times \text{VD})/\text{Cl}$ (RIVIERE, 1997). For example, variation of total body water may be introduced by age or hydration status, which will affect VD and can increase or decrease $t_{1/2}$ accordingly; or perhaps, an identified increase in protein binding limits VD and contributes to a direct decrease of $t_{1/2}$ (KIMBLE *et al.*, 2013). Alternatively, increased Cl induced by glomerulonephritis will decrease $t_{1/2}$; hepatic perfusion changes slowing drug metabolism will protract it.

It is not entirely predictable which effect will predominate, although some drug $t_{1/2}$ alterations are very clearly the pathologic impact on VD or CI (ABO-EL-SOUD and GOUDAH, 2010). In some disease processes, neither VD nor CI change appreciably until well into the terminal stages of disease, or more frustratingly, the same disease concern exerts different effects depending on the species (RIVIERE, 1983). In other situations, both independent parameters are affected and exert a blended effect on $t_{1/2}$. However, for the clinician faced with a dosing regimen in a clinical body system, this formula serves as reminder of important considerations when setting the initial dose and for adjustment of this dose following patient response.

Utilisation of a well-conducted pharmacokinetics study may begin through the development of a treatment plan or identify potentials of maximal efficacy and minimal toxicity. During an actual treatment course, therapeutic drug monitoring can be utilised with context of such studies to adjust an unsuccessful treatment regimen. In any of these situations, it is important to recall that the plasma concentrations, rather than the given dose of the drug, are the more important points of reference for the patient. Furthermore, signalment within the study may be widely different from the patient, if for no other reason than study participants are usually healthy. Lastly, these studies are not ones of direct assessment of pharmacodynamics, except in a few instances where effects can be assessed practically (CARPENTER *et al.*, 2012). It, therefore, is critical to understand that apparent lack of toxicity neither implies safety nor efficacy. Although in the end, truly no replacement exists to direct pharmacokinetic assessment for a given drug in its intended species (PAGE and PAPICH, 1997; RIVIERE, 1997; TOUTAIN *et al.*, 2010). Identification of the important components for such studies within the literature will give the practitioner maximal benefit from the studies conducted to date.

Logistics – pharmacokinetic primer through literature review

From the likely sources for exotic animal pharmacokinetic publications, 106 studies were reviewed from 2010 - 2014 (FOOTNOTE). Over 30 % of these publications were focused on species which could be defined as production-driven, such as racing camels, aquaculture species, or game birds (TOUTAIN *et al.*, 2010). As frequent companion animals, reptiles and psittacines each represented 20 %, and from wildlife rehabilitation efforts, raptors and marsupials each contributed 10 % of the remaining publications. The other 24 investigations studied marine mammals, elephants, and non-psittacine avian species in four or more publications each; while non-human primates and non-production hoofstock were represented minimally; and carnivores and amphibians were absent.

Production incentive aside, this literature showed a skew to the current medications in vogue, problem-solving for clinical challenges, and taxon specific issues. Unsurprisingly, long-acting antibiotics were featured strongly as they both are desired for convenient clinical treatment regimens and have marked interspecies variability that requires dose verification (BERTELSEN *et al.*, 2010; ADKESSON *et al.*, 2011; BAKKER *et al.*, 2011; GARCÍA-PÁRRAGA *et al.*, 2011; WOJICK *et al.*, 2011; ADKESSON *et al.*, 2012; HOPE *et al.*, 2012; DECHANT *et al.*, 2013; MEEGAN *et al.*, 2013; CHURGIN *et al.*, 2014; NARDINI *et al.*, 2014; STEEIL *et al.*, 2014). Ability to provide analgesia to avian patients effectively and with minimal handling began before this review (PAUL-MURPHY *et al.*, 2009) and remains a challenge through the current investigations (SANCHEZ-MIGALLON GUZMAN *et al.*, 2013; CLANCY *et al.*, 2014). Management of aspergillosis for penguins progressed through the current antifungal availability, and identified concerns of compounded product inefficacy (BUNTING *et al.*, 2009; BECHERT *et al.*, 2010) and toxicity resulting from pharmacokinetic taxon-specificity (HYATT *et al.*, 2014). Similarly, persistent treatment failures of the Australian icon, the koala, for chlamydiosis produced a series of studies that generally concluded gastrointestinal physiology and marked hepatic biotransformation dictated by the *Eucalyptus* diet complicated pharmacokinetics for most standard pharmaceuticals, often rendering them ineffective at the current dose recommendations (GRIFFITH *et al.*, 2010; GOVENDIR *et al.*, 2012; BLACK *et al.*, 2013; BLACK *et al.*, 2014).

Due to the challenges of exotic animals as patients, let alone study participants, not one article in this review can be cited as entirely complete for inclusion of all ideal components. However, much of the research incorporates many of the considerations, and provides discussion when limitations were presented. As these complications also will present in daily practice, it is appropriate to consider all the facets in a conjoined review with cited examples for reader assessment.

Route and formulation

Specific to the study drug, consideration to route of administration is required in context of the real-life use or experimental approach. Whenever possible, an intravenous assessment for the drug in the target animal is critically important (RIVIERE, 1997). When toxicities are probable, or unknown, or compounding would be required to create an intravenous product, these studies would be considered inappropriate, especially in the exotic animal arena. However, it is from this route that distribution and clearance parameters are best estimated. Furthermore, this route is required for interpretation of bioavailability for extravascular routes, although estimations may be made through some additional calculations or perhaps by using published intravenous studies.

For extravascular routes, several considerations must be made. Although oral dosing by gavage ensures full dose administration in the study, rarely would this approach be utilised in a clinical situation. Pharmacokinetics resulting from direct oral administration may not be equivalent for within feed presentation (MILLER *et al.*, 2012; FLAMMER *et al.*, 2013). For some drugs, fasting versus feeding can impact a drug's availability (BECHERT *et al.*, 2010; RIVERA *et al.*, 2012; WACK *et al.*, 2012). For some species, their interaction with food by tearing versus swallowing intact can alter the systemic manipulation of the intended dose (HARMS *et al.*, 2011; WACK *et al.*, 2012). Parenteral extended dose depositions can be affected markedly and inconsistently by interspecies variability. In particular, changes in peripheral fat reserves can be detected as prolonged absorption interval or drug persistence (GARCÍA-PÁRRAGA *et al.*, 2011). Variation in skin thickness – or perhaps from grooming which introduces an enteral route – may alter the persistence of topical products (TOUTAIN *et al.*, 2010; CARPENTER *et al.*, 2012). Environmental manipulation should be considered for ectotherms with temperatures of enclosures or basking spots (PREZANT *et al.*, 1994; GAUNT *et al.*, 2012; SUN *et al.*, 2014), or application of bath agents to aquatic animals as possible combination of administration routes (YANG *et al.*, 2014). Pharmacokinetic analyses can be strengthened by incorporation of these variables into the planned study approach.

As additional consideration for formulation, it must be determined in what version the compound is available. Commercial products have the benefit of quantifiable analysis to ensure dose administered; although differing lot numbers or bottle sources could introduce a study variable (KILBURN *et al.*, 2014), this issue is unavoidable in larger specimens. Compounded products may seem imminently customisable to study needs. However, repeated assessment of professionally compounded products or those compounded at patient side from commercial products have demonstrated concerns to both study results and ultimate clinical application so must be validated before administered dose can be presumed (SMITH *et al.*, 2010; EMERY *et al.*, 2012; SOUZA *et al.*, 2012).

Timing, sample, and analysis

To provide sufficient data points for a given study, timing of sampling should be considered from previously published studies for initial plan development. However, impacts of work day, expected animal compliance, and variation between collaborators must be accommodated to the final schedule. Inherent in this decision is that of single- or multi-dose regimens. Although many studies are published with a single route of only a single dose of the given drug, interpretation beyond the study is enhanced by multiple routes at a single dose (MOLTER *et al.*, 2013). Additionally, these studies then may give rise to basis for repeated dosing studies (FREDHOLM *et al.*, 2013; DELK *et al.*, 2014).

It should be considered if only blood-based samples will be collected for the study. Destructive sampling for the study or planned tissue harvest following euthanasia may serve an important means

to identify penetration of the given dose or could identify toxicity (GAMBLE *et al.*, 1997; FANG *et al.*, 2013; DELK *et al.*, 2014; SUN *et al.*, 2014). However, even when not planned, if an animal dies within the study time period, opportunistic banking of tissues should be performed, minimally for future analysis. In any situation with extravascular parenteral drug dosing, administration sites are important tissues for collection. Other intended sampling sites should be considered based on the drug's intended action and analysis of non-blood fluids, such as urine, faeces, or tears, could elaborate on the pharmacokinetics with little increase in patient risk (FREEMAN *et al.*, 2013).

Many techniques of pharmaceutical analyses are available and often are the funding limitation to these studies. It is important to target the techniques to the drug in question and to not exclude the potential for known active metabolites in other species (RIVIERE, 1997). For standardisation, comparison of experimental samples to a curve created from the target species plasma or serum is most ideal for minimising interspecies confusion to interpretation. However, plasma or serum collection prior to study commencement for this naïve banking will affect the overall study duration if the same individuals are utilised. Consideration for previously banked serum samples could reduce this impact to the study individuals and timeline.

Pilot studies

Often overlooked, the pilot study often is an essential part in effective pharmacokinetic preparation (RIVIERE, 1997). By processing the full methodology as a smaller preliminary trial, dose administration specifics can be determined; timing of sampling confirmed; and planned analytical method verified. When successful, the resulting data can be incorporated into the main study profile and may increase available participants. When unsuccessful, pilot studies save unnecessary sampling; time the sample collection most effectively to real-life schedules; and prevent wasted funds if the presumed analysis identifies problems with sample processing or drug measurement. When very limited numbers of animals are available, each individual may serve as its own pilot study which may lengthen the study duration or introduce inter-assay variability as samples cannot be batched. An alternative approach for a controlled pilot study has been published as use of a prior clinical patient with intermittent sample collection from a therapeutic drug monitoring perspective.

Sample sizes

For exotic animal investigations, management of available sample sizes is key to a successful study. Unlike domestic animal protocols where many individuals are available, zoo and wildlife species may not even have statistically sufficient numbers within a given captive population. Even through collaboration of multiple institutions, variability in management sufficiently can affect study parameters that the actual study becomes separate smaller trials. Available groups recruited opportunistically from ill populations can introduce further limitations to sample size, although it is the targeted group for the drug and presents realistic context. Generally, however, healthy animals are the sample source for these investigations.

When numbers of participants are small (< 5 definitively, and 5 - 10 for consideration), it is more appropriate to provide the raw data for the time and plasma concentration curves and pharmacokinetic analysis by individual (RIVIERE, 1997). Benefits of this approach include the ability to assess variability, and need for fewer individuals, which as a result, present fewer logistical issues, reduced cost, and quicker time for project completion. Disadvantages of these lower numbers are that the study is statistically less robust, and a gender or age bias to the study group may preclude identification of these issues' impact (CHINNADURAI *et al.*, 2014).

To increase a study group number, many investigators have incorporated larger groups of animals, but not as true populations. For species which do not tolerate repeated handling or are sufficiently iconic so have political pressure to minimise handling for research (FREEMAN *et al.*, 2013; MEEGAN *et al.*, 2013), and animals with body size simply too small to provide sufficient volume (CHINNADURAI *et al.*, 2014), sparse sampling from a group of individuals may provide a complete assessment over the

time needed to complete the study. Incumbent to this approach is the blunted or lost ability to identify variation for a given participant. However, it does reduce impact of the study to that particular animal from handling or sampling.

Naïve pooled or naïve averaged data each have been presented successfully within the literature (FREEMAN *et al.*, 2013; MEEGAN *et al.*, 2013; CHINNADURAI *et al.*, 2014). Where the data is pooled, all plasma concentrations at any given time point are used and assessed with the assumption that it is from one animal. As such, this analysis is affected minimally by time variation so animal non-compliance will have lower impact. Similarly, data averaging also presumes to model a single animal from group data, although individual variability is lost entirely as plasma concentrations are averaged for an averaged time point to create the curve. These manipulations are not true population assessments, which require very large sample numbers and more complex sampling and modeling considerations (KUKANICH, 2011), particularly when disease impact is figured into the sample size (RIVIERE, 1983). At this time, it is not entirely clear which grouped approach is best for the exotic animal situation. It may be defined by the animals available, and analysis of determined results by each methodology may prove the better approach to confirm most appropriate data presentation (MEEGAN *et al.*, 2013).

Ethics - in studies and practice

Perhaps those studies that opportunistically recruit clinical patients (GRIFFITH *et al.*, 2010; GOVENDIR *et al.*, 2012; INNIS *et al.*, 2012; BLACK *et al.*, 2013; FREEMAN *et al.*, 2013; MEEGAN *et al.*, 2013) as the study population are more ethically comfortable than those with healthy animal participants. In this situation, the animals must receive treatment so potential benefit of recovery from the drug is present. Routinely, veterinarians are collecting repeated diagnostic blood samples from these animals. Therefore, the proscribed evaluation of the outcome can be integrated into the treatment protocol and pharmacokinetics considered retrospectively. Provided consideration is made for the standard limitations of 1 % by body weight for blood sampling, a strong case could be made for these groups as allowable, and even optimal, for pharmacokinetic studies. In fact, for some species, this approach may be the only means to evaluate pharmacokinetics agreeably to the animals' management. However, in either this situation or the study with healthy animals, the baseline blood collections for standardisation curves would originate from healthy animals.

Other types of study populations are exemplified in the pharmacokinetic literature. Purpose driven or repeated study colonies are not infrequent (PAUL-MURPHY, *et al.*, 2009; SANCHEZ-MIGALLON GUZMAN *et al.*, 2010; SANCHEZ-MIGALLON GUZMAN *et al.*, 2011; EMERY *et al.*, 2012; SOUZA *et al.*, 2012; SANCHEZ-MIGALLON GUZMAN *et al.*, 2013; GUSTAVESSEN *et al.*, 2014; SANCHEZ-MIGALLON GUZMAN *et al.*, 2014). These animals may be subjected to multiple courses of not only administered drugs but also blood collection, although it is a consideration that they serve to spare other individuals from these analyses to a broader group benefit. Acquisition of a colony specifically for a pharmacokinetic trial has been made with similar consideration for the collective good. This intended selection can be ameliorated potentially by end of study adoptions (CHURGIN *et al.*, 2014). It must be stated, however, that a colony with an end-point of euthanasia – perhaps even a requirement unrelated to the pharmacokinetic trial (GAMBLE *et al.*, 1997) – can be maximised to an even greater extent than the non-terminal study to assess tissue drug concentrations, systemic toxicity, or injection site complications (DELK *et al.*, 2014). Leaving the debate of a study's subjects, the methodology must be considered for ethical completion of these projects. Handling parameters of anaesthesia or manual restraint must consider that anaesthetics are themselves drugs and can affect pharmacokinetics of the study medication. Subsequent to handling, actual collection of the sample via catheter or intermittent venipuncture requires consideration, and may be more appropriate ethically in different situations dependent on clinical experience or available facilities. Those individuals which can be managed by operant conditioning for venipuncture represent an enviable ideal for minimally invasive pharmacokinetic

investigations. Inclusion of appropriate facets of a well-constructed study as discussed previously should be foremost in consideration. Concurrent appropriate monitoring for intended – and unintended – effects permits prompt management of participant issues and exclusion from data interpretation.

It may not be possible to convince some colleagues to the contrary that the healthy exotic animal could – and should – participate in a pharmacokinetic trial. To those clinicians, it is challenged in return to be aware that every day in a typical zoo or wildlife practice, an unevaluated drug is administered to a patient (RIVIERE, 1997) – perhaps by their own hand. Is this not unfounded pharmacokinetic research? At worst, it could be construed that an extralabel drug administration has more detriment here than in a pharmacokinetic trial. From these non-validated treatments, it is unclear that the drug is efficacious, let alone safe. For an animal that needs treatment, it actually could be considered as withholding optimal care. At best, it is a lost opportunity to uncover physiologic peculiarities. In the end, it should be clear that the benefit to a clinical patient from the pharmacokinetic studies performed, including those in healthy animals, is great. Furthermore, it is incumbent to understand data management of these studies to apply their conclusions not only ethically but also successfully whether the clinician is the investigator or prescriber.

Conclusion

In closing, it is encouraged that pharmacokinetics be considered in the true spirit of alchemy. The base manipulation of the data is uncomplicated and can provide adjustment to an unsuccessful treatment or identify a point for directed clinical management. By taking the mundane measurements of time and plasma drug concentrations, a clinician creates a golden opportunity for progressive veterinary care.

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FOOTNOTE: Listing of all citations reviewed for this plenary talk and its accompanying presentation, but not necessarily cited specifically, can be received by contacting the author by email.

THE USE OF HALOPERIDOL PREMEDICATION TO REDUCE CAPTURE STRESS DURING ANAESTHESIA OF SPOTTED DEER (*AXIS AXIS*) WITH XYLAZINE AND KETAMINE

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This study assessed the efficacy of oral haloperidol premedication in reducing stress during capture of captive spotted deer (*Axis axis*). Deer were premedicated with haloperidol tablets (Serenace 10[®], RPG Life Sciences Ltd., Ankleshwar, Gujarat, India) at a dose of 1 mg/kg body weight, administered in bananas. Six hours post-administration, deer were darted with a combination of xylazine (Ilium Xylazil-100[®], Troy Laboratories Pty. Limited, Glendenning, NSW, Australia) at the dose of 3 mg/kg body weight and ketamine (Ketamil[®], Troy Laboratories Pty. Limited, Glendenning, NSW, Australia) at the dose of 2 mg/kg body weight. At the end of the anaesthetic procedure, yohimbine (Reverzine[™], Bomac Pty. Limited, Hornsby, NSW, Australia) was administered at the dose of 0.3 mg/kg body weight divided equally and administered IV and IM. Rectal temperature and heart rate were monitored at 5 minute intervals. Blood was obtained via jugular venipuncture immediately after induction and 35 minutes post-induction. Blood gas analysis was performed and serum cortisol was determined only on the 35 minute sample. Repeated measures variables were analysed with a 2-way ANOVA and paired samples were analysed with the Student's *t*-test. Significance was determined at $p \leq 0.05$. Heart rate, rectal temperature, blood lactate and serum cortisol were all significantly higher in non premedicated deer; whereas, venous pH was significantly lower (table 1).

Tab. 1: Comparison of parameters between non premedicated and premedicated deer.

Groups	Heart rate* (beats/min)	Rectal temperature* (°C)	Blood lactate* (mmol/L)	Venous pH*	Serum cortisol** (µg/dL)
Non premedicated	83 ± 28	40.7 ± 0.9	9.25 ± 4.65	7.26 ± 0.07	2.83 ± 1.21
Premedicated	66 ± 7	39.7 ± 0.89	6.88 ± 3.91	7.32 ± 0.04	1.81 ± 0.95

(* 0 minutes post-induction, ** 35 minutes post-induction)

Lower heart rate, rectal temperature, blood lactate and pH following premedication were indicative of decreased activity during induction. Lower serum cortisol indicated decreased capture stress with premedication. Haloperidol premedication proved to be beneficial at reducing capture stress in these deer.

THE USE OF PARTIAL-OPIOID ANTAGONISM COMBINED WITH OXYGEN INSUFFLATION TO SUPPORT THE PHYSIOLOGY OF CHEMICALLY IMMOBILISED WHITE RHINOCEROS (*CERATOTHERIUM SIMUM*)

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White rhinoceros (*Ceratotherium simum*) immobilised with a combination of etorphine, azaperone and hyaluronidase experience adverse physiological changes including severe hypoxia, hypercapnia, respiratory and metabolic acidosis and tachycardia. Immobilisation of rhinoceroses is a necessary and integral part of conservation efforts both in zoos and the wild. However, this procedure places an animal at risk and may result in morbidity and mortality.

The goal of this study was to identify the best available intervention(s) to improve the cardiorespiratory function of immobilised white rhinoceros. Eight sub-adult male white rhinoceroses were captured in Kruger National Park and taken to Bomas where they were housed for the duration of the experiment. Boma kept animals were used in order to limit confounding variables. After a period of Boma adaptation, all the rhinoceroses were immobilised with a combination of etorphine (3 mg, M99, Novartis, Kempton Park, South Africa), azaperone (45 mg, Stressnil[®], Janssen Pharmaceutical Ltd., Halfway House, South Africa) and hyaluronidase (2500 i.u. lyophilised hyalase, Kyron Laboratories, Benrose, South Africa) on four occasions at two-week intervals so that each rhinoceroses received the same four experimental interventions in a randomised order. The experimental interventions, administered at 6 min after lateral recumbency, included various combinations of butorphanol (45 mg, Kyron Laboratories, Benrose, South Africa) and oxygen insufflation via nasotracheal intubation. Oxygen was administered at a constant flow rate of 30 L/min. The partial opioid-antagonists butorphanol was administered at 15x the etorphine dose. A control experiment was also conducted in each rhinoceros whereby no supportive treatment was given after immobilisation. Physiological measurements including heart rate, respiratory rate, blood pressure, haemoglobin oxygen saturation, and arterial blood gas samples were taken at 5 min intervals throughout a 20 min immobilisation period.

The findings of this study will be presented to show the effects of the various treatment interventions and how they are best suited to support an immobilised rhinoceros' cardiorespiratory physiology. The critical adverse effects of using oxygen insufflation on its own will be highlighted and the practical use of the other interventions will be discussed.

LONG-TERM ANAESTHESIA FOLLOWING TWO DIFFERENT INDUCTION PROTOCOLS IN SOUTH AFRICAN IMPALA (*AEPYCEROS MELAMPUS*)

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In order to develop a long-term anaesthesia for flighty antelope species in field situations, two different protocols followed by maintenance of anaesthesia with an intravenous constant rate infusion (CRI) were evaluated in impalas (*Aepyceros melampus*). In a cross-over study, 10 adult female impalas were induced with two different induction protocols: 0.2 mg/kg medetomidine (MEDETOMIDINE 10 mg/ml; Kyron; Benrose; RSA) + 4 mg/kg ketamine (KETAMINE 100 mg/ml; Bodene; PE; RSA) + 0.15 mg/kg butorphanol (BUTORPHANOL 10 mg/ml; V-TECH; Pretoria; RSA) (MKB) and 0.375 mg/kg etorphine (CAPTIVON 98; 9,8 mg/ml; Wildlife Pharmaceuticals; White River; RSA), + 0.2 mg/kg medetomidine + 0.2 mg/kg midazolam (DORMICUM 15 mg/ 3 ml; Roche; Randburg; RSA) (EMM). Anaesthesia was maintained with a CRI at an initial dose rate of medetomidine (1.2 µg/kg/h) + ketamine (2.4 mg/kg/h) and midazolam (36 µg/kg/h). Reversal was achieved with naltrexone (TREXONIL 50 mg/ml; Wildlife Pharmaceuticals) (2:1 mg butorphanol; 20:1 mg etorphine) and atipamezole (ATIPAMEZOLE 50 mg/ml; V-TECH; Pretoria; RSA) (5:1 mg medetomidine). Evaluation of anaesthesia included respiratory rate, heart rate, rectal temperature, arterial blood pressure, oxygen saturation, end tidal carbon dioxide tension and tidal volume every 10 min, palpebral reflex and response to painful stimuli every 15 min and arterial blood gases every 30 min. EMM resulted in a faster induction and recovery but also produced significant respiratory depression, hypoxaemia and respiratory acidosis, whereas MKB induced animals maintained stable values for the entire observation period. The CRI provided surgical anaesthesia for 120 minutes in 7/10 animals (MKB) and 9/9 animals (EMM) respectively.

PHARMACOKINETICS OF SINGLE DOSE INTRAVENOUS AND ORAL FLUNIXIN MEGLUMINE IN THE BLACK RHINOCEROS (*DICEROS BICORNIS*)

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Zoological medicine requires clinicians to trust that drug doses and routes, which have been proven safe and effective in model domestic species, will function equivalently in their exotic patients. This approach often occurs with minimal to no research for validation in the target species. Ideally, definitive pharmacokinetic analysis of medications would be available prior to the treatment of exotic species to diminish potential side-effects and maximise desired results.

In captive black rhinoceros (*Diceros bicornis*), a variety of medical conditions are treated using anti-inflammatories with doses based on domestic equine models or anecdotal reports. To establish species specific pharmacokinetics, flunixin meglumine was administered once at 1 mg/kg intravenously (FlunixiJect™, Butler Schein Animal Health, Dublin, Ohio, USA) and orally (Banamine® Paste, Merck Animal Health, Whitehouse Station, New Jersey, USA) to two male black rhinoceroses with doses separated by a minimum of two weeks. Serial blood samples were collected at predetermined time points to create a plasma concentration versus time curve which was analysed by non-compartmental methods to describe pharmacokinetics for this drug. Intravenously, flunixin meglumine had an elimination half-life of 27.6 h for one animal but only 4.8 h for the second. Orally, it reached mean C_{max} (362.5 ng/ml) at 2.9 h, and had elimination half-life of 4.4 h and 6.2 h. Mean oral bioavailability was 58.3 %. Compared to similar pharmacokinetic studies in horses, oral flunixin meglumine had lower bioavailability and C_{max}, and a slightly longer elimination half-life.

CAPTURE OF FREE-RANGING WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*) WITH A COMBINATION OF MEDETOMIDINE, AZAPARONE AND ALFAXALONE

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The addition of alfaxalone to medetomidine and azaparone significantly reduced induction time while sparing cardiopulmonary function in white-tailed deer (CAULKETT *et al.*, 2014). Nine female white-tailed deer (*Odocoileus virginianus*) were captured for radio collar placement in Grand Forks, British Columbia, Canada. Medetomidine (M) (Medetomidine 30 mg/ml, Bow Valley Research, Calgary, Alberta, Canada) was administered IM, via a Dan-Inject[®] darting system, at an estimated dose of 0.15 mg kg⁻¹, azaparone (AZ) (Stresnil[®], Vetoquinol, Lavaltrie, Quebec, Canada) at an estimated dose of 0.2 mg kg⁻¹ and alfaxalone (AL) (Alfaxan[®], Abbott Laboratories, Ltd, Saint-Laurent, Quebec, Canada) at an estimated dose of 0.5 mg kg⁻¹. Time to sternal recumbency (mean \pm SD) was 7.85 \pm 1.7 min. All animals induced to sternal recumbency with one dart. The actual drug doses received were: M (0.12 \pm 0.03 mg/kg), AZ (0.16 \pm 0.14 mg/kg) and AL (0.31 \pm 0.11 mg/kg). Three of nine deer required additional drugs to induce lateral recumbency, as they had experienced dart failure, with a partial injection. Time to lateral recumbency was 13.1 \pm 8.4 min. Recovery was smooth and uneventful following the IM administration of atipamezole (Atipamezole 10 mg ml⁻¹, Bow Valley Research, Calgary, Alberta, Canada) at 5 times the medetomidine dose. Time to head lift was 7.9 \pm 1.7 min, and deer were standing in 9.9 \pm 1 min. The major side effects of the combination were hypoxaemia, evidenced by a mean PaO₂ of 48 \pm 4.7 mmHg, and hypoventilation (PaCO₂ of 61 \pm 3.4 mmHg). Induction was very calm, the mean lactate (obtained 15 - 30 min post darting) was 0.39 \pm 0.17 mmol/L, and rectal temperature ranged from 37 to 38.5°C. Hypoxaemia and hypoventilation are commonly encountered in deer anaesthetised with medetomidine-based combinations. Supplemental inspired oxygen is advised to counter hypoxaemia. This combination can be used to successfully capture free-ranging white-tailed deer.

Reference

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COMPARISON OF TWO SIMILAR ANAESTHETIC PROTOCOLS FOR PROLONGED RECUMBENCY USING ISOFLURANE AND INTERMITTENT BOLUS INJECTION OF ETORPHINE IN AN ADULT MALE AFRICAN ELEPHANT (*LOXODONTA AFRICANA*)

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Summary

An adult male African elephant (*Loxodonta africana*) had to be anaesthetised twice for tusk extraction due to chronic suppurative pulpitis. On both occasions the animal was sedated with detomidine and azaperone. Anaesthesia was induced with etorphine and acepromazine and maintained with isoflurane, etorphine and acepromazine. Recovery was slow after the first procedure. Therefore on the second occasion lower isoflurane and higher etorphine doses were used for anaesthesia maintenance. This led to an improvement in anaesthetic quality and ventilation, and faster recovery.

Introduction

Anaesthetic management of adult elephants for prolonged recumbency is challenging due to their massive size and mechanical considerations relative to recumbency (STEFFEY, 2006). We describe minor modifications made to the initial protocol to improve the anaesthetic quality in an adult male African elephant (*Loxodonta africana*) that had to be anaesthetised twice.

Case descriptions

A 17 year old, 4,550 kg, male African elephant had to be anaesthetised twice for tusk extraction due to chronic suppurative pulpitis. On the first occasion the elephant was sedated with 100 mg detomidine (Domosedan[®], Orion Corporation, Espoo, Finland) i.m. and 200 mg azaperone (Stressnil[®], Sanachemia Pharmazeutika, Neufeld, Austria) i.m. and anaesthesia was induced 25 min later with 7.7 mg etorphine and 37.5 mg acepromazine (Large Animal Immobilon[™], Novartis Animal Health UK Ltd, Herts, UK) i.m. The animal was positioned in right lateral recumbency on inflatable inner tubes of truck tires. The trachea was intubated with a 45 mm ID and 1.8 m long endotracheal tube using the stylet technique (ZUBA and OOSTERHUIS, 2012). Anaesthesia was maintained with 0.5 - 0.8 % isoflurane (vaporizer setting) (Attane[™], Provet AG, Lyssach, Switzerland) administered in oxygen and air via two large animal anaesthetic machines joined in parallel at the Y-piece (DUNLOP *et al.*, 1988). The

flow rates of oxygen varied between 14 and 19 L/min and air between 2 and 9 L/min. 80 ml lidocaine 2 % (Lidocain 2 % Chassot, Vétoquinol AG, Ittigen, Switzerland) was injected locally around the tusk root. Seventy min after induction of anaesthesia supplemental boluses of 0.245 mg etorphine and 1 mg acepromazine (= 0.1 ml LA Immobilon™) were given i.v. every 15 min. Heart rate (HR) remained stable between 35 and 45 bpm and respiratory rate (RR) between 5 and 6 bpm throughout the procedure. Blood pressure was measured invasively using an ear artery. Forty min after starting isoflurane inhalation, mean arterial pressure (MAP) decreased from 90 mmHg to 50 mmHg. Therefore the isoflurane concentration was decreased from 0.8 to 0.5 % and dobutamine (Dobutrex, Teva Pharma AG, Basel, Switzerland) CRI was initiated (0.3 µg/kg/min). After 50 min MAP increased to 85 mmHg and the dobutamine CRI was stopped. In total, 45 L of crystalloid fluids was given i.v. over 160 min resulting in a fluid rate of 3.7 ml/kg/h. Additional fluids were given rectally using a hose with constant slow running warm water. Due to technical difficulties, end-tidal CO₂ could not be measured. Instead arterial and venous blood gas analyses (BGA) were performed every 20 to 40 min. As the portable analyser did not function properly due to the cold ambient temperatures, it was not possible to measure all the parameters at every time point. The results of the BGA are summarised in table 1. The BGA results revealed mild hypercapnia from the onset of inhalation anaesthesia and severe hypercapnia about 160 min after induction of anaesthesia. Despite attempts to manually ventilate the elephant, severe hypercapnia (PaCO₂ 90 mmHg) and acidosis (pH 7.2) persisted. It was therefore decided to abort the procedure. Anaesthesia was reversed with 100 mg atipamezole (Antisedan®, Orion Corporation) i.m. and 450 mg naltrexone (Naltrexone 50 mg/ml, Kyron Laboratories, Benrose, South Africa) (½ i.m. and ½ i.v.) 190 min after administration of induction agents. Recovery was slow. The animal seemed weak and required several attempts to stand. He was standing after 44 min, but was severely ataxic and did not properly use the dependent front limb. The animal showed trembling over the whole body for several hours. Post-operative analgesia included 0.8 µg/kg buprenorphine (Temgesic®, Reckitt Benckiser Healthcare LTd, Slough, UK) i.m. and 0.12 mg/kg meloxicam (Metacam 20 mg/ml, Boehringer Ingelheim GmbH, Basel, Switzerland) s.c. Meloxicam was given the first time two days before surgery and then repeated daily p.o.

Six months later the elephant was anaesthetised again. The animal was sedated with 110 mg detomidine and 220 mg azaperone i.m. Once the elephant was in standing sedation, a specially designed net, which was connected to a crane, was attached to the elephant. Anaesthesia was induced with 9.8 mg etorphine and 40 mg acepromazine i.m. 50 min after administration of the sedative agents. The animal was positioned in left lateral recumbency. The trachea was intubated and 0.4 - 0.5 % isoflurane was administered in oxygen and air. Compared to the first occasion, lower isoflurane concentrations were used from the beginning and the isoflurane inhalation was discontinued 50 min prior to reversal. To ensure adequate depth of anaesthesia, i.v. boluses of 0.245 mg etorphine and 1 mg acepromazine were started 58 min after induction. The boluses were given every 10 min and increased to every 7 min after discontinuation of isoflurane. Dobutamine CRI was administered from the onset, initially 0.5 µg/kg/min and then decreased to 0.3 µg/kg/min after 2 hours. In total, 53 L of crystalloid fluids were given i.v. over 180 min resulting in a fluid rate of 3.9 ml/kg/h. Additional rectal fluids were given as described above. Heart rate remained between 35 and 45 bpm, RR between 5 and 6 bpm and MAP between 80 and 105 mmHg throughout the procedure. End-tidal CO₂ varied from 40 to 61 mmHg. The pulse oximeter probe was attached to the ear and values ranged from 92 to 98 %. Arterial blood gas analyses were performed every 20 to 30 min. The results of the BGA are shown in table 2. The animal showed mild acidosis and hypercapnia starting 70 min after induction until the end of the procedure. Every 30 min the elephant's limbs were massaged. Forty-five min prior to reversal, the anaesthetic machines were disconnected and the Mega-Vertebrate Demand Ventilator was connected to the endotracheal tube to assist spontaneous breathing. The portable demand ventilator is manually triggered and provides large tidal volumes using compressed oxygen.

Anaesthesia was reversed 190 min after induction with 150 mg atipamezole i.m. and 309 mg naltrexone (½ i.m. and ½ i.v.). Recovery was fast and smooth and the bull was standing within 20 min with no signs of ataxia. The same analgesic protocol was given as for the first occasion.

Tab. 1: Results of arterial and venous blood gas analyses performed during the first procedure.

Results of venous analyses are shown in italics.

Parameter	T50	T70	T85	T115	T160	T190	T210
pH	7.284	7.262	7.267	7.2	7.184	7.197	7.203
PaCO ₂ (mmHg)	71	57	64		93	87	90
PvCO ₂ (mmHg)				<i>84</i>			
PaO ₂ (mmHg)	233	191			265		135
sO ₂ (%)	100				100		98
Bicarbonate (mmol/L)		25.4	29.4	32.5	34.9	33.9	35.2
Lactate (mmol/L)	2.09	1.01					

T represents the time in minutes after administration of induction agents.

Tab. 2: Results of arterial blood gas analyses and corresponding end-tidal CO₂ values performed during the second procedure.

Parameter	T55	T75	T90	T110	T140	T180	T200
pH	7.319	7.263	7.244	7.234	7.235	7.287	7.287
PaCO ₂ (mmHg)	43	52	52	77	70	56	52
EtCO ₂ (mmHg)	49	51	58	60	57	50	53
PaO ₂ (mmHg)	100	129	172	204	229	135	102
sO ₂ (%)	97	98	99	99	100	99	97
Bicarbonate (mmol/L)	22.3	23.7	22.3	32.7	29.5	26.6	24.6
Lactate (mmol/L)	11.8	4.8	9.9	3.0	3.5	9.3	1.6

T represents the time in minutes after administration of induction agents.

Results and discussion

The main modifications of the second anaesthetic procedure, which led to an overall improvement of the anaesthetic quality, can be summarised as follows. By using lower doses of isoflurane combined with higher doses of etorphine ventilation was improved, resulting in less severe hypercapnia and acidosis. With the discontinuation of isoflurane 50 min prior to reversal, there seemed to be no residual effect of isoflurane at recovery allowing for a faster, smoother recovery with no ataxia. The lower doses of isoflurane combined with low dose dobutamine CRI ensured an adequate blood pressure throughout the procedure. The use of a net combined with the crane allowed for easier positioning of the animal and this allowed us to place proper padding beneath the dependent limbs preventing nerve injury. Lastly the second procedure took place during warmer ambient temperatures and the portable blood gas analyser and monitoring equipment functioned better.

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CHALLENGES WITH CAPTURE AND ZOLAZEPAM-TILETAMINE IMMOBILISATION OF FREE-RANGING AND CAPTIVE GREY WOLVES (*CANIS LUPUS*)

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Capture and drug administration are potentially stressful events for free-ranging and captive wildlife; and wolf captures are especially challenging. We evaluated physiological effects of capture and immobilisation in grey wolves (*Canis lupus*) immobilised for radio-collaring in the wild and for eye examination at a zoo in Sweden. Free-ranging wolves (26 - 57 kg) were darted from a helicopter with zolazepam-tiletamine (ZT) (Zoletil forte vet., Virbac S.A., Carros, France) at a total dose of 500 mg ($n = 22$) or 250 mg ($n = 5$). Captive hand-raised wolves (35 - 41 kg) were immobilised by hand injection of 170 mg ZT ($n = 5$). Serial arterial blood samples were collected anaerobically and immediately processed with a portable analyser. No marked alterations in pulmonary gas exchange or acid-base status were identified in the captive wolves, whereas mild hypoxaemia (PaO_2 60 – 79 mmHg) was recorded in one captive and nine free-ranging wolves. Lactic acidaemia, hypocapnia, and hyperthermia were recorded in free-ranging wolves following physical exertion during the induction period. Side effects recorded during ZT immobilisation included salivation, retching, tachycardia, and poor muscle relaxation. Due to body movements during immobilisation, medetomidine (Domitor[®] vet., 1 mg/ml, Orion Pharma Animal Health, Turku, Finland) was administered intramuscularly by hand to all captive and 13 free-ranging wolves at a total dose 0.3 - 1.5 mg, followed by atipamezole (Antisedan[®] vet., 5 mg/ml, Orion Pharma Animal Health) at five times the medetomidine dose at the end of the procedure. Recoveries were prolonged, even with the lower ZT doses, and ataxia was observed up to seven h after initial ZT injection. In conclusion, helicopter darting and ZT immobilisation of wolves can lead to physiological alterations and prolonged recoveries.

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ALFAXALONE ANAESTHESIA IN THE BENGALESE FINCH (*LONCHURA DOMESTICA*)

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A three-way cross over design was used to assess the effects of 10, 30 and 50 mg/kg of alfaxalone (Alfaxan, Jurox) administered subcutaneously to ten Bengalese finches (*Lonchura domestica*). Landmark induction and recovery times were recorded and depth of anaesthesia was assessed throughout. The highest dosages were associated with significantly quicker induction times, compared with the lower dosages (10 mg/kg; 311 ± 203 seconds, 30 mg/kg; 216 ± 87 seconds, 50 mg/kg; 111 ± 32 seconds). Similarly, recovery was dose dependent with animals receiving 10 mg/kg standing at 29 ± 4 minutes, 30 mg/kg standing at 65 ± 17 minutes and 50 mg/kg standing at 93 ± 19 minutes. Oxygen saturation (MouseOx Plus, Starr Life Sciences Corp.) at 15 minutes was approximately 96 % for all birds, with heart rates ranging from 701 – 900+ (measurements were limited to 900 bpm due to apparatus limitations). A trend towards lower heart rates was seen with higher dosages. Respiratory rates were variable and showed no clear pattern between protocols. Subjects were rousable throughout, deep pain responses were not lost at any dose and muscle relaxation was dose dependent and generally poor. The lowest dosage did not result in a working level of anaesthesia, however 30 and 50 mg/kg would facilitate non-invasive procedures. Investigations have subsequently been carried out with 30 mg/kg alfaxalone combined with medetomidine, midazolam or butorphanol with the aim of improving muscle relaxation and decreasing deep pain responses for applications in passerines when gaseous anaesthesia is not available.

EVALUATION OF A NOVEL SUSTAINED-RELEASE FORMULATION OF DOXYCYCLINE IN PARROTS (*PSITTACULA KRAMERI*) AND IN DOMESTICATED CHICKENS (*GALLUS GALLUS DOMESTICUS*)

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Avian chlamydiosis, caused by the bacteria *Chlamydia psittaci*, affect many orders of birds but particularly *Psittaciformes* which are most susceptible to infection by this pathogen. The disease is zoonotic and when it affects flocks of birds it may cause economic losses. The drugs of choice for treatment of this disease belong to the tetracycline group, especially doxycycline but also oxytetracycline, given intramuscularly every 2 to 5 days (depends on the drug used) or orally every 24 hours for 45 days. This treatment involves a significant and prolonged stress for the bird but also frustration and high expenses for the owner and therefore results in low level of compliance by bird owners. In this study, a long-acting formulation of doxycycline (Doxycycline hyclate, Sigma-Aldrich Ltd., Rehovot, Israel) dissolved in-situ gelation system based on a thermo-sensible polymer, Poloxamer 407, was tested in rose-ringed parakeets (*Psittacula krameri*) and in domesticated light-breed chickens (*Gallus gallus domesticus*). The aim of the study was to maintain a minimal inhibitory concentration (MIC) of 1 µg/ml doxycycline in the serum, which is an efficient level against *Chlamydia*, for at least 21 days following a single injection dose. Twenty-four parrots and 34 chicks were injected subcutaneously once, and levels of doxycycline in the serum were measured during 42 - 49 days in predetermined times by Liquid chromatography – Mass spectrometry method. The average concentrations of doxycycline stayed above MIC for 12 days in the parrots but 21 days in the chickens. An erosive skin reaction was developed at the injection site, however it was dissolved spontaneously and further treatment was not necessary. Future studies are needed in order to improve the formulation for prolonging the duration above MIC for more than 21 days along with minimising the local reaction in the injection site.

OCCURRENCE OF *BABESIA* SPECIES IN CAPTIVE REINDEER (*RANGIFER TARANDUS*) IN GERMANY

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Two cases of acute babesiosis occurred in captive reindeer (*Rangifer tarandus*) in German zoos in 2009 and 2012, respectively. Since nothing thus far was known about the abundance and geographic distribution of *Babesia* species in reindeer in Germany, the aim of this study was to investigate the occurrence and species diversity of *Babesia* parasites infecting reindeer in different zoos and deer parks in Germany. Between June and December 2013, blood samples were taken from around half of the country's reindeer population (123 animals from 16 different facilities). The samples were tested for the presence of *Babesia* species DNA by conventional PCR and partial sequence analysis of the 18S rRNA gene. In addition, Giemsa-stained blood smears were examined for parasitaemia by light microscopy.

The overall PCR-prevalence in blood samples was 23.6 % ($n = 29$). Comparison of sequenced amplicons with GenBank entries revealed four different *Babesia* species: *B. venatorum* ($n = 19$), *B. capreoli* ($n = 6$), *B. odocoilei*-like ($n = 2$) and *B. divergens* ($n = 1$). One sample revealed 100 % homology to *Theileria* sp. Only four out of the 16 facilities were tested entirely negative. Examination of Giemsa-stained blood smears was less sensitive, but intraerythrocytic *Babesia* parasites were detected in samples of three reindeer from three locations. Since all tested animals were clinically healthy and parasitaemia was low, the infections were probably chronic and persistent.

The high prevalence of *Babesia* infections implicates babesiosis to be a relevant health threat for captive reindeer in Germany. Whenever reindeer in German zoos or deer parks show clinical signs like fever, anaemia, haemoglobinuria or jaundice, acute babesiosis should be taken into account and the animal should immediately be tested either by PCR or by examination of Giemsa-stained blood smears.

THE GREAT APE HEART PROJECT: A MODEL FOR COLLABORATIVE DISEASE INVESTIGATION

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Cardiovascular disease (CVD) has been identified as a major cause of death in captive great apes. The Great Ape Heart Project (GAHP) based at Zoo Atlanta, (www.greatapeheartproject.org) has designed an innovative and coordinated programme to investigate ape CVD and establish uniform, state of the art, cardiac diagnosis, therapeutic, and prevention strategies for great ape CVD. The GAHP is endorsed by the Association of Zoos and Aquariums (AZA) management groups for all four ape taxa.

A top priority for the GAHP has been to develop a database that ensures that all archived and prospective CVD-relevant clinical and pathology data are compiled into a confidential yet searchable resource. This effort was awarded a 2012 Institute of Museum and Library Services (IMLS) National Leadership Grant that funded the design and implementation of the software platform and is currently in beta testing with several zoological institutions within the USA. This tool provides for best practices for collection and management of CVD-relevant data. It improves clinical management by allowing more targeted and timely reports to individual institutions regarding their animals. Ape cardiac necropsy protocols are being standardised and the database will allow for comparison between clinically relevant information and pathologic information at necropsy. The database supports statistically robust studies, involving numerous subject matter experts (SME's), to encourage discovery of potential causes of ape CVD. The GAHP offers its resources to veterinarians, cardiologists and other SME's beyond the United States, working in Canada, Mexico, Europe, and Africa. The information that can be generated and collected in a coordinated and cohesive manner, from ape collections with varied genetic and husbandry backgrounds could prove to be very valuable in the search for potential aetiological factors leading up to ape CVD.

The effort to investigate ape CVD in Europe is known as the (European) Ape Heart Project. Based at Twycross Zoo, it is directed by Sharon Redrobe (EAZA great ape TAG vet advisor) and led by Victoria Strong (clinical veterinary PhD student, University of Nottingham) and involves a number of SME's from a variety of disciplines. Endorsed by the European Association of Zoos and Aquaria (EAZA) Great Ape Taxon Advisory Group (GATAG), the programme incorporates a combination of retrospective epidemiological review and prospective clinical and pathological studies. The programme encourages zoos to perform routine cardiac assessment on their great apes as part of health checks. European zoos performing such examinations are asked to follow the guidelines available on the programme website (see <http://www.twycrosszoo.org/ape-heart-project.aspx>) and submit their findings to the GAHP database for analysis by the European group. Additionally, in the event of a great ape death, the European programme requests zoos to examine and remove the heart as per a basic protocol and, after fixing, submit it whole for (free of charge) detailed gross and histopathological examination. The European projects aim is to identify patterns of disease by correlating the findings of both clinical assessment and *post mortem* examination. The collaboration between these two ape heart projects, based in America and Europe, will promote further understanding of heart disease and improve captive great ape veterinary care worldwide.

SURGERY: OLD AND NEW TECHNIQUES APPLIED TO WILD ANIMALS

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Surgery in the zoological collection setting presents a unique set of challenges, as well as opportunities. A small published evidence base, and the low surgical case volume in a zoological collection, leads to a high uncertainty level as to likely outcomes. Zoo surgery also presents opportunities, with interest from specialist veterinary and human surgeons, and medical companies, which may allow the application of novel technologies and techniques. Minimally invasive surgical techniques, such as laparoscopy, arthroscopy, interventional radiology, and minimally invasive plate osteosynthesis, hold particular potential advantages in zoo animals, with small wounds, reduced morbidity, and an immediate return to social groups and normal activity often possible. Other recently developed technologies such as locking orthopaedic plating systems, absorbable orthopaedic components, 3D printed models and patient specific implants, new designs of joint replacements, robotic surgery, nanotechnology, biological meshes, and even transplants of stem cell repopulated de-cellularised organ matrixes, raise interesting new possibilities in zoological surgery, as well as needing special ethical considerations as to their application and case selection. While lower in media profile, recent advances in improving surgical safety and outcomes in human surgery can also be extremely useful when applied in the high uncertainty environment of zoological surgery. Outcome auditing, surgical checklists, and other techniques, can all help optimise surgical decision making, animal welfare, and post-operative outcomes in a zoological collection.

SURGICAL MANAGEMENT OF A PERSISTENT RIGHT AORTIC ARCH IN A GIRAFFE (*GIRAFFA CAMELOPARDALIS*)

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A 5 month old, female giraffe presented with a 3.5 month history of occasional milk, regurgitation progressing to food regurgitation. Oesophageal endoscopy revealed a stricture at the level of the heart base, suggesting a vascular ring anomaly. One month later, the animal was anaesthetised with 2.5 mg/kg ketamine and 0.084 mg/kg medetomidine (both ZooPharm, Wyoming, USA). A left intercostal incision was made parallel to the caudal border of the triceps, but the leg provided significant barrier to heart visualisation. Visualisation was increased through traction of the limb, requiring two to four people to rotate as their strength tired. The latissimus dorsi was incised, the serratus muscle ray divided, and pleura incised to visualise the persistent right fourth aortic arch. The ductus arteriosus originated from the left subclavian artery-aortic arch junction and entered the main pulmonary artery causing oesophageal stricture. The ductus was friable making ligation difficult and ductus haemorrhage could not be controlled. Further surgical exposure was not possible without removing the forelimb, thus necessitating euthanasia. This procedure may have been successful if performed in a younger giraffe, as the thorax would be smaller and the foreleg may have more available motion.

Alternatively, partial caudal scapulectomy with permanent removal or plate and screw fixation may offer improved exposure of the heart base. In this case, recovery was not performed but recovery in a padded stall of a transport trailer was planned. Thoracic drains, common in other species, would not have been utilised as the postoperative care would have been limited.

ATTEMPTED SURGICAL MANAGEMENT OF AN INTRAMEDULLARY SPINAL TUMOUR IN A BABIRUSA (*BABYROUSA BABYRUSSA*)

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Cases of hind limb paralysis and ataxia are relatively common in small animal medicine; however their management in a zoo setting can prove to be an immense challenge. Our case report refers to a 14 year old, cc. 50 kg, female babirusa who we had been treating for signs of epilepsy over a five year period, presented with a sudden onset of bilateral, hind limb paralysis presumably following a seizure. Similar clinical signs had previously been seen after such an episode, thus it was initially presumed that the condition would resolve following treatment with corticosteroids and supportive therapy as such was our former experience. As clinical picture failed to improve, five days after the onset of the symptoms a Computed Tomography (CT) scan was performed. As general anaesthesia for the procedure, the following protocol was implemented: 25 mg zuclopenthixol acetate (Cisordinol-Acutard 50 mg/ml, Lundbeck Hungaria, Budapest, Hungary), 125 mg tiletamine/zolazepam (Zoletil 50, Virbac laboratories, Carros, France) + 60 mg azaperone (Stresnil, Janssen Pharmaceutica N.V., Beerse, Belgium), and 5 %, then down to 4 % sevoflurane (Sevoflurane Baxter, Baxter Hungary, Budapest, Hungary) for maintenance. A non-contrast CT scan of thoracolumbar spine (T3 - L3), with a single-slice helical CT scanner (GE-e), revealed an intramedullary swelling of the spinal cord at the level of the T12 vertebra with complete loss of epidural fat and myelocompression from the dorsal aspect. Based on these alterations immediate surgical intervention was carried out. The surgery was initiated presuming that anatomically, the spinal cord of the babirusa was similar to that of the domestic pig. For the procedure, the patient was placed in a sternal recumbent position and the anatomical site in question was approached from the dorsal aspect. The muscle layers overlying the spinal column were transected, the multifidus muscle was pulled away from the spinous processes in both directions and a dorsal laminectomy was performed. In the intramedullary area, a uniform pathological mass was observed coinciding with the CT findings. This mass was most probably responsible for the compression of the spinal cord and the cause of the clinical signs presented. The alteration was removed from the epidural space and was sent for histopathological investigation. Results revealed a high-grade, non-differentiated tumour of neuroepidermal origin with a large number of apoptotic figures and a high mitotic index. Postoperative treatments consisted of pain management, general fluid therapy and energy supplementation over the course of a 2-week period as further life-threatening symptoms were not observed. Furthermore, physiotherapy was also initiated in the patient five days post-operatively in order to prevent muscular atrophy. Despite our intense efforts, after two weeks, since only minor improvements were noted in the animal, she was humanely euthanised for welfare reasons.

A FASCIOCUTANEOUS FLAP FOR COVERAGE OF EXPOSED BONE IN THE HIND LIMB OF A ROTHSCHILD GIRAFFE (*GIRAFFA CAMELOPARDALIS ROTHSCHILD*)

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A young, hand-reared Rothschild giraffe (*Giraffa camelopardalis rothschildi*) sustained a serious wound below its right hock following a suspected traumatic injury. This resulted in severe lacerations with soft tissue loss and an area of exposed metatarsal bone.

Initial treatment with antibiotics (3 g amoxicillin, BetamoxLA, Norbrook, Newry, UK and 1.1 g enrofloxacin, Baytril Max, Bayer, Newbury, UK) and wound dressings were unsuccessful in resolving the injury, which started to worsen.

Four weeks after the initial injury the giraffe was anaesthetised for surgery using 9 mg medetomidine i/m (Zalopine 10 mg/ml, Orion Pharma, Turku, Finland), 150 mg ketamine i/m (Vetalar-V 100 mg/ml, Pfizer Ltd, Sandwich, UK) and then isoflurane (Isoflurane-Vet, Merial Animal Health Ltd, Harlow, UK). A random patterned pedicled fasciocutaneous flap was raised and rotated to cover the skin defect. A split thickness skin graft was then taken from the thigh to cover the donor defect. Finally the graft donor site was covered with a dressing.

The wound required intensive management. Initially this consisted of dressing changes every 48 h, performed aseptically under anaesthesia with extensive wound flushing with iodine solution (Povidone-iodine 7.5 %, Animalcare Ltd, York, UK). As the wounds healed the inter-dressing interval increased. Problems encountered during this period included partial wound breakdown and antibiotic resistance.

After five months the wound had healed enough that all treatment stopped. However, after a further four months minor trauma to the wound required further, less intensive, medical management including dressing changes for seven weeks.

Over eleven months the giraffe underwent 71 anaesthetics, mainly with medetomidine and ketamine and reversal with atipamazole, with no adverse effects.

This report illustrates that, although problems were encountered, it is possible to undertake complex surgery, such as skin grafts, in large zoo animals with successful results.

ENDOSCOPIC SALPINGECTOMY IN NON-HUMAN PRIMATES

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Endoscopy in non-human primates (NHP) has resulted in improvements for both research and clinical applications. Sterilisation-using endoscopy is a necessity with species where self-mutilation of surgical wounds is common. Endoscopic salpingectomy was performed on six Crab-eating macaques (*Macaca fascicularis*) and six Barbary macaques (*Macaca sylvanus*) living at the Refuge de l'Arche, France. They were anaesthetised with Ketamine 5 mg/kg body weight IM (Ketamine 1000[®], Virbac Pharma Animal health, Carros, France) in combination with Medetomidine 0.05 mg/kg body weight (Domitor[®], Orion Pharma Animal health, Espoo, Finland). After induction, all animals were intubated and maintained with Isoflurane, a general physical examination was performed. To prior to an endosurgery procedure an abdominal ultrasonography scan was performed to evaluate the reproductive track. All pregnant animals and animals that had endometriosis were excluded from the procedure. Only one out of thirteen female primates was pregnant. The animals were presented in dorsal recumbency for the procedure. Following aseptic preparation, a 3 mm skin incision was made in the midline, just 2 cm caudal to the umbilicus. A Veress needle was inserted through the *Linea Alba*. After that, the abdominal cavity was inflated with carbon dioxide to a pressure of 12 mmHg. The Veress needle was removed, and a sharp trocar cannula for the telescope was inserted through the same opening. The 2.7 mm telescope housed within its protection sheath was inserted in the cannula until the abdominal cavity. Organs were visible on the monitor. The placement of the sharp trocar cannula was done by transillumination with the telescope. When making the stab incision for the trocars, large vessels could be easily identified and avoided. The incisions were performed between the umbilicus and the pelvis on both sides and equidistant to the midline. A second, 3.5 mm sharp trocar cannula was inserted through a 2 mm skin incision. The incision and the insertion of the cannulas were viewed directly on the monitor. A third trocar was applied in the same manner. A 3 mm curved Kelly forceps was inserted in the second trocar through the cannula and was used to locate the fallopian tubes by gently lifting the uterus. The fallopian tube appeared as a delicate thin pink structure and the ovary as a white granulate structure. We used a 3 mm bipolar coagulating forceps to cauterise a fallopian tube. After cauterisation, the bipolar forceps were withdrawn and Metzenbaum endoscopic scissors were inserted to cut a section of at least 1 cm in length of the tube. Only the tips of the scissors were used so that the resection was carefully monitored. The resected section was removed from the abdominal cavity via the cannula. The surgical sites were observed to ensure adequate haemostasis. No complication associated with self-mutilation of surgical wounds was observed and healing time was significantly reduced. Salpingectomy is preferred to ovariectomy, to maintain the ovaries hormone production, to decrease incidences of haemorrhage and inflammation of the severed ends of the fallopian tubes and the associated reproductive organs and to reduce surgical times. However, laparoscopic tubal sterilisation reversal was theoretically possible in NHP, as it was in humans.

DENTIGEROUS CYST IN THE NARES OF A JUVENILE WHITE CROWNED MANGABEY (*CERCOCEBUS ATYS LUNULATUS*) TREATED SUCCESSFULLY BY MINIMAL INVASIVE SURGERY

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An eight months old male, hand-reared, white crowned mangabey (*Cercocebus atys lunulatus*) presented with intermittent right unilateral nasal discharge and epistaxis. An initial rhinoscopic examination did not reveal any evident lesions, so the assumption of an essential epistaxis, a condition well described in humans, was made. A few months later, keepers reported a mass in the left nostril. A second examination confirmed a large space occupying mass in the left nasal cavity. Biopsy of the mass indicated a fibropapilloma. The MRI of the head of the patient showed a lesion distorting much of the nasal cavity and the nasal septum without invading it. Minimal invasive surgery assisted by rhinoscopy could then be scheduled. Medetomidine (50 µg/kg IM; Medetor[®], Virbac, France, Carros, France) and Ketamine (5 mg/kg IM; Kétamine1000[®], Virbac France) were used as inductive agents and Isoflurane in oxygen (Aerrane[®], Baxter S.A.S, Maurepas, France) was used for maintenance. Analgesia was insured with Meloxicam (0.2 mg/kg IM; Métacam[®], Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany). After intubation, the patient was placed in dorsal recumbence, the head elevated and the oropharynx packed with moistened gauze, taking care not to impede the movement of fluid from the nasopharynx into the mouth, while flushing. Meanwhile and prior to passing the scope, the patient's nose was decongested and anaesthetised using topical sprays (oxymetazoline (Déturgylone[®], Sanofi-Aventis France, Paris, France) in small gauzes first, followed by lidocaine spray (Xylocaïne10%[®], AstraZeneca, Rueil-Malmaison, France). The nasal cavities were then flushed using warm sterile saline to remove any debris and excess mucus. A rigid, sheathed nasal endoscope was used in order to enable intraoperative flushing to maintain visualisation. Endoscopy helped to identify the absence of any connection between the septum, which is highly vascularised, and the mass. The septum was deviated on the opposite side. As the mass was completely separated from the septum, rigid and attached to the anterior lateral part of the nasal fossa, instrumental luxation of the superior and external attachments of the mass presented low risk of haemorrhage. Ablation of the mass was not followed by any bleeding, so no electro cautery was used though the instruments were prepared in case of accidental haemorrhage. The integrity of the nasal septum was confirmed by post-operative rhinoscopy. Final histological analysis revealed a dentigerous cyst, for which the complete excision should be curative. Attentive analysis of the preoperative MRI images showed the connection of the dentigerous cyst to the anterior tooth. One-year post surgery, the animal is healthy and no sign of discomfort around the nares is seen.

SUCCESSFUL TREATMENT OF A SARCOID IN A SOMALI WILD ASS (*EQUUS AFRICANUS SOMALIENSIS*) USING ALDARA® 5 % CREAM (IMIQUIMOD)

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Sarcoids are the most common skin neoplasms in horses and donkeys. They have also been described clinically in wild equids including Somali wild ass. Classic treatment options described in domestic horses include ligation, surgical or laser removal, cryotherapy and local immune modulation (Bacillus Calmette-Guerin) therapy, amongst others. Newer and promising treatments in domestic equids include the use of intra-tumoral bioabsorbable cisplatin beads/emulsion, autologous implantation and the use of topical creams containing acyclovir or imiquimod (Aldara® 5 % cream, 3M Health Care Limited, Loughborough, England).

Simon, a 19 year old Somali wild ass stallion, presented with an ulcerated mass on the left side of his face. Under standing sedation with detomidine 0.08 mg/kg (Domosedan® 10 mg/ml solution for injection, Elanco Animal Health, Basingstoke, England) and butorphanol 0.1 mg/kg IM (Dolorex® 10 mg/ml solution for injection, MSD Animal Health, Milton Keynes, England) punch biopsies were obtained for histopathology and a blood sample was collected. Blood work was unremarkable but the histopathology confirmed the mass to be a sarcoid.

Several treatment options were considered including surgical excision, use of topical cytotoxic therapy (AW4 cytotoxic cream "Liverpool cream") and use of topical Aldara® 5 % cream. Therapeutic challenges included dealing with an untrained animal, the need for long term post-operative care if a surgical procedure was chosen and the practical and occupational health considerations of using a cytotoxic cream. In contrast, the option of using non-toxic Aldara® cream, applied by the keepers every three days and causing minimal inflammation and discomfort was very attractive, improving the chances of treatment compliance.

Aldara® 5 % cream was applied every three days to the affected area for a period of two months. Analgesia with phenylbutazone (Pro-Dynam® oral powder, Dechra Veterinary Products Ltd, Shrewsbury, England) was used on two occasions during treatment due to a change in demeanour but in general treatment was well tolerated. Treatment had to be restarted two months later for a period of one month but after this the sarcoid regressed completely. No evidence of recurrence has been noted to date (seven months since cessation).

In this case, Aldara® 5 % cream (imiquimod) proved a feasible and effective treatment option for sarcoid in a wild equid when other more aggressive options were not possible.

DIAGNOSTIC IMAGING OF NASAL NEOPLASIA IN ASIATIC BLACK BEARS (*URSUS THIBETHANUS*)

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Summary

Many health problems are recorded in captive bears (BOURNE, *et al.*, 2010), but the rescued population of ex-farm bears at Animals Asia's Chengdu Bear Rescue Centre (www.animalsasia.org) demonstrate a spectrum of pathology not fully reported in this species elsewhere in captivity. This presentation will document the radiographic and computed tomography (CT) findings in a case series of nasal carcinomas in four Asiatic black bears (*Ursus thibethanus*) in China. Additional cases in Europe and Vietnam will also be referenced.

Introduction

It is estimated that around 12,000 Asiatic black bears (*Ursus thibethanus*) are incarcerated in bile farms across China (LOEFFLER *et al.*, 2009). Despite the recognition of bear farming, along with poaching, trade and deforestation as major threats to this species (GARSHELIS and STEINMETZ, 2008) and the classification of Asiatic black bears as "vulnerable" by the IUCN, commercial farming to extract bear bile persists.

Bear bile has been used sparingly in traditional Chinese medicine for over 2,000 years (LI, 2004) not just in China, but in countries across Asia. However, in the commercial farming process, bile is extracted from live bears through the creation of a painful and non-sterile surgical fistula (BACON, 2008; LOEFFLER *et al.*, 2009), leading to a plethora of physiological and psychological pathologies.

Collaboration with human physicians working in hospitals in Chengdu allowed staff from the Animals Asia Chengdu Bear Rescue Centre to perform computed tomography (CT) imaging techniques to aid diagnosis. CT imaging in this species for diagnosis of nasal carcinoma has not yet been described.

To date, six Asiatic black bears rescued from bear bile farms in China and Vietnam by Animals Asia have been diagnosed with nasal carcinomas, and subsequently euthanised. This presentation will introduce the clinical signs, diagnostic imaging techniques and diagnosis of four of the six cases.

Materials and methods

As part of Animals Asia's rescue, rehabilitation and public education work, and in collaboration with

local government agencies, Asiatic black bears are seized from bear bile farms across China and Vietnam and transferred to an Animals Asia sanctuary. At the sanctuaries, bears are triaged, health problems identified and treatment plans developed. Each bear undergoes rigorous assessment and both medical and behavioural evaluation before intensive medical treatment and psychological rehabilitation can begin.



Fig. 1: An adult male Asiatic black bear (Ursus thibetanus) arriving at the sanctuary in a typical farm cage. Cranial alopecia is obvious. (Photo: Bacon HJ)

Each bear in the series presented in slightly different ways. Diagnostic imaging techniques were only available for bears residing at the Chengdu sanctuary in China.

Tab. 1: Demographics of bears affected with nasal carcinomas.

Bear ID	Gender	Location	Comments	Imaging
A	Male	China	Adult, newly arrived from bear farm	Dental radiography and CT
B	Female	China	Geriatric	CR-Radiography and CT
C	Female	China	Adult	CR-Radiography and CT
D	Female	China	Adult	CR-Radiography and CT
E	Male	Vietnam	Adult	None
F	Female	Vietnam	Adult	None

The first case diagnosed was an adult male with cranial alopecia and unilateral muco-purulent discharge on arrival from the farm (see figure 1). All other cases presented in bears residing at one of the sanctuaries. Four of six bears presented with nasal discharge although this varied from serous to

muco-purulent and was often intermittent. Discharge often became haemorrhagic as the disease progressed. Facial deformities were not evident in any of the Asiatic black bears. Behavioural signs of pain were the most common indicators of pathology but developed insidiously. Behavioural signs included decreased activity, increased resting, head pressing against a hard surface, stereotypy, reduced appetite and pressing a paw to the face.

All bears were initially treated with antibiotics based on culture and sensitivity of nasal discharge, and either carprofen (Rimadyl, Zoetis, London, UK) (4 mg/kg SID PO) or meloxicam (Metacam, Boehringer Ingelheim, Bracknell, UK) (0.1 mg/kg SID PO) where indicated. If behavioural signs of pain persisted despite non-steroidal-anti-inflammatory drugs, then tramadol, HCl 50 mg, local manufacturer, (2 - 4 mg/kg BID PO) or Buprenorphine (Sogeval, York, UK) (0.01 - 0.02 mg/kg TID IM) were also administered to deliver multimodal analgesia. Tumours often seemed to present with waxing and waning symptoms, with bears varying in demeanour and activity over a period of weeks, making assessment based on clinical signs difficult. Persistence of symptoms after appropriate antibiotic and analgesic therapy resulted in anaesthesia for full clinical examination.

Weight was estimated based on previous weight records or experience. Anaesthesia was induced with 500 mg tiletamine-zolazepam (Zoletil 100, Virbac, Bury St Edmonds, UK) reconstituted with 5 ml medetomidine, (Domitor, 1 mg/ml, Zoetis), to create a solution of approximately 100 mg/ml tiletamine-zolazepam and 1 mg/ml medetomidine and 1 mg/kg of tiletamine-zolazepam in combination with 0.1 mg/kg medetomidine injected into the left tricep using a jabstick (safety stick pole syringe, Jorvet, Loveland, USA). This anaesthetic combination has been successfully used in a variety of bear species, although doses vary. Once induced, bears were intubated with a 16 mm or 18 mm endotracheal tube, stabilised and maintained on 1 - 3 % isoflurane (Isoflo, Abbot, Maidenhead, UK) and oxygen, and the medetomidine reversed with a half dose of atipamazole (Antisedan, Zoetis) to reduce cardiovascular effects.

All bears were examined for dental abnormalities and a full physical examination performed. Palpation of the facial bones and hard palate revealed no abnormalities or asymmetry and no abnormalities were detected on retropulsion of the globes. Repeated bacteriological and fungal culture were performed from samples of both the right and left nasal cavities using bacteriological transport swabs. For bear A, radiography, (which was limited due to the equipment available in the rescue centre), of the nasal cavity was undertaken using a dental radiography head and kodak film at 2.5 sec, 8 amps and 70 kV. All other bears based in the China sanctuary were radiographed using donated digital Computer radiography (CR) (Fuji FCR Capsula XL). Bears based in the Vietnam sanctuary (E and F) were not imaged due to a lack of radiography equipment, but were diagnosed based on clinical signs, *post mortem* and histopathology as euthanasia due to welfare concerns was performed under anaesthesia.

In all bears located in China, further investigations were decided and the bears were transported to a local human hospital for computed tomography (CT) assessment of the lesions seen on radiography. Delivery of isoflurane and oxygen was discontinued and maintenance of anaesthesia for transportation and CT was achieved with intermittent 0.2 - 0.5 ml intravenous boluses of tiletamine-zolazepam-medetomidine as indicated by assessment of anaesthetic depth based on lingual pulse rate, respiratory rate, jaw tone and palpebral reflex. The endotracheal tube was retained in place.

CT images were obtained using a standard human CT tube. Bone scan window settings were selected and the nasopharynx was imaged with the bear positioned in dorsal recumbency.

Nasal biopsies were taken using biopsy forceps introduced into the nasal cavity via the nares. Once investigations were complete, each bear was transported back to the rescue centre and for bear B, atipamezole 0.06 mg/kg i.v. was administered. Bears A, C and D were euthanised directly under anaesthesia and necropsies were performed immediately. Bear B was recovered as to this point its

clinical signs had been mild, but due to non-compliance with medication consistent with winter dormancy, it was later euthanised.

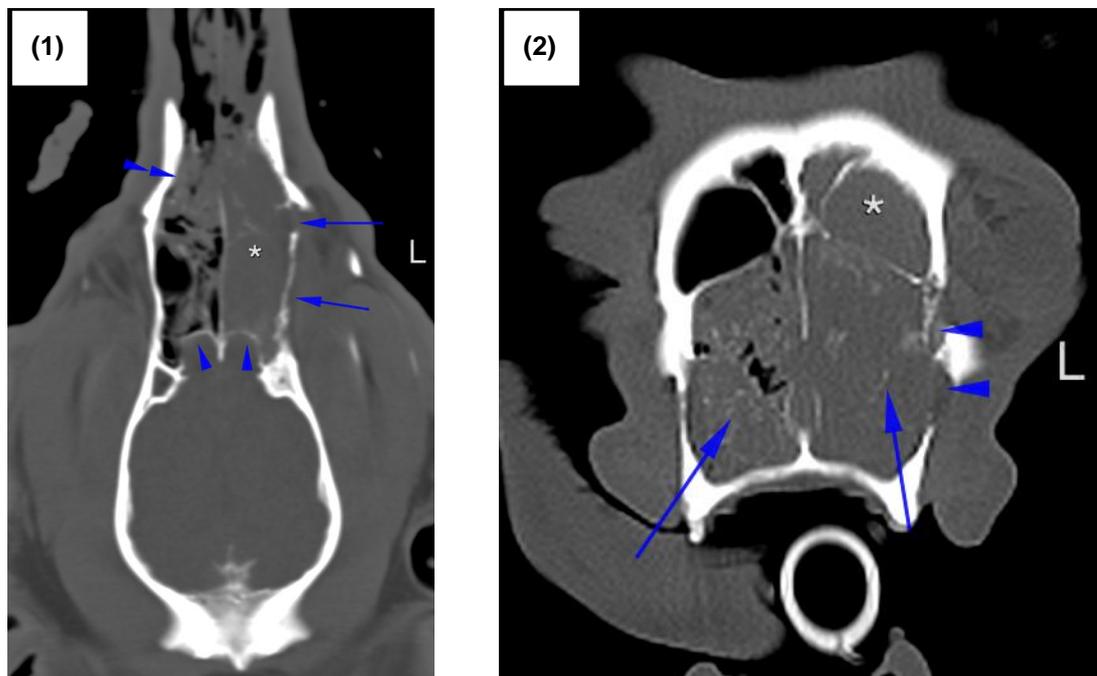


Fig. 2: CT images of Bear A showing (1) dorsal view and (2) saggital view of the nasal cavities. The tumour is marked with * and indicated by blue arrows.
(Photos: © Animals Asia)

Results and discussion

In this case series, radiography, both digital CR and film radiography using a dental X-ray machine was useful in establishing turbinate destruction, but gave limited information as to mass invasion and destruction of surrounding tissues. This case series indicates that CT scanning of the head is a practical and effective tool in the diagnosis of nasal carcinomas in this bear species, and should be considered in all cases with a waxing and waning activity level and nasal discharge, for early diagnosis. The waxing and waning nature of the disease may be as a result of concurrent infection and/or analgesic administration.

Tab. 2: Summary of CT results for four bears with nasal carcinomas.

Bear ID	Cribiform plate destruction	Gross extension into calvarium and mass effect	Osteolysis (maxilla, nasal septum)	Extension into frontal sinus	Extension into nasopharynx
A	Y	N	Y	Y	Y
B	Y	Y (mild)	Y	Y	Y
C	Y	Y	Y	Y	Y
D	Y	Y	Y	Y	Y

Four bears confirmed with nasal adenocarcinoma, 1/6 confirmed with adenosarcoma and 1/6 confirmed with nasal squamous cell carcinoma.

The exact cause or causes of cancer in bears are unknown. A number of different neoplasias are recorded in bears (BOURNE *et al.*, 2010). Neoplasias in farmed bears often involves the hepatobiliary and gastrointestinal systems with up to 35 % of bile farmed Asiatic black bears at the Chengdu sanctuary dying from hepatic cancer, though other tumours are also recorded. The aetiologies of ursid neoplasias are likely to be varied as in other species, with genetic and environmental factors such as infection, inflammation, trauma, nutrition and toxins all contributing.

Nasal adenocarcinomas have a number of aetiologies such as infectious e.g. enzootic nasal adenocarcinoma in sheep and goats (DE LAS HERAS *et al.*, 1991), or environmental such as exposure to leather, textile or woodworking industries in humans (ZHENG *et al.*, 1992; CALDERÓN-GARCIDUEÑAS *et al.*, 2000; HEMELT *et al.*, 2004).

Histopathology is required for definitive diagnosis of nasal adenocarcinoma, but CT imaging can provide extremely useful indicators of nasal cancer in all species. CT imaging may give more accurate information regarding the extension of tumours into surrounding structures than radiography (THRALL *et al.*, 1989). CT has been shown to be more sensitive (88 %) than radiography (72 %) for determining the location and extent of nasal lesions in dogs and cats (BARREAU, 2008). Most nasal tumours are malignant and are characterised by progressive local invasion with destruction of bony structures. Approximately 30 % of dogs with epithelial tumours suffer from metastasis into the brain (BARREAU, 2008). At initial diagnosis clinically detectable metastases are only present in 0 – 12 % of dogs (BARREAU, 2008). Metastatic disease was not noted in any of these cases but was recorded by the author in a nasal carcinoma case in a European brown bear (PERSONAL COMMUNICATION).

Therapy of nasal tumours generally involves radiation (MELLANBY *et al.*, 2002; GIEGER *et al.*, 2008), is palliative rather than curative and aims to extend quantity and quality of life. Surgery alone is not a treatment option for nasal tumours (BARREAU, 2008) and neither surgery nor chemotherapy have been significantly associated with survival time in dogs (HENRY *et al.*, 1998).

In the presented cases of nasal tumours, radiation therapy was not available, practicable, or ethically justifiable and so palliation using multimodal analgesia was applied until definitive diagnosis was achieved. Multimodal analgesia lead to a significant improvement in behavioural clinical signs compared to single use of NSAIDS, but efforts should be made to seek a diagnosis as quickly as possible and euthanasia should be considered prior to reduction in quality of life.

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EQUINE SARCOID IN SOMALI WILD ASS (*EQUUS AFRICANUS SOMALICUS*): PRELIMINARY REPORT OF A SURVEY IN THE EUROPEAN ENDANGERED SPECIES PROGRAMME (EEP)

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The equine sarcoid (ES) is the most common equine skin neoplasia. Several cases of ES in Somali wild asses in the EEP (190 individuals in 35 institutions on 31.12.2014) were reported to the coordinator. Therefore, an ongoing survey was initiated in 2011 with the aim of investigating samples (biopsies, surgeries, and *post mortem* examinations) of suspected skin lesions in this species.

We received eight samples from five European countries: six of Somali wild asses (*Equus africanus somalicus*), one of an onager (*Equus hemionus*), and one of a zebra (*Equus zebra hartmannae*).

Histology confirmed the diagnosis with ES-typical dermal proliferation of spindle-shaped fibroblasts for all cases. Bovine papillomavirus type 1 (BPV1) DNA was detected in four of the Somali wild ass samples, in the onager and in the zebra sample using PCR. Equine papillomavirus type 2 DNA was detected in one wild ass and one was negative for papillomavirus DNA by PCR.

These findings suggest that BPV1-associated equine sarcoids are present in the European captive Somali wild ass population and other wild equids. This is the first report of BPV1-associated ES in onagers, whereas for zebras this was previously published.

BPV1 and BPV2 have been identified as being causally involved in ES. The major histocompatibility complex class haplotype can be an additional risk factor for the development of these tumours. A genetic predisposition would of course have serious implications for breeding programmes of captive wild equids. Therefore, the captive Somali wild ass population has to be carefully evaluated regarding ES in accordance with ongoing research activities in domestic equids.

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NONE-SURGICAL ARTIFICIAL INSEMINATION IN FELIDS: ASIATIC GOLDEN CAT (*CATOPUMA TEMMINCKI*) AND PERSIAN LEOPARD (*PANTHERA PARDUS SAXICOLOR*)

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Summary

Here we report an artificial insemination (A.I.) approach in Asiatic golden cats (*Catopuma temmincki*) and Persian leopard (*Panthera pardus saxicolor*), which was modified to overcome the difficulties of cervical canal passage in felids. With this non-surgical method, we achieved three litters of healthy cubs twice in an Asiatic golden cat (AGC) and once in a Persian leopard (PL) during a total of 4 trials (3 x AGC, 1 x PL). During the examination, we were able to collect basic data on the reproduction physiology of these species. While in the U.S. assisted reproduction techniques in felids have been intensely studied for several species, this is the first time these methods were successfully applied in European zoos.

Introduction

The success rate for artificial insemination (A.I.) in non-domestic felids is reported to be below 20 % (SWANSON, 2009). Nevertheless may A.I. be desirable to supplement captive breeding programmes of highly endangered felids. Several factors contribute to the difficulties with feline A.I.:

- Inaccessibility of most species;
- Lack of knowledge on reproduction physiology (e.g. ovarian cycle duration breeding periods);
- Semen collection and ejaculate quality of the male;
- Detection/ induction of oestrus and timing of A.I.;
- Induction of ovulation;
- Influence of anaesthesia/ stress on the female.

A major problem is the incompatibility of supposed breeding partners, which may result in fatal injury of the female. The latter was the reason for A.I. in the cases reported here.

Material and methods

A female Asiatic golden cat (born 2006, weight: 9 kg) and 0,1 Persian leopard (born 2005, 35 kg) were subjected for A.I. In the AGC, two different males were used as semen donors (1,0 Lao, born 2004, weight 14.7 kg and 1,0 Hotan, born 2003, weight: 13.7 kg); the male PL was born in 2004 and weighted 55 kg. Both females were nulliparous during the first AI trial.

The cat keepers recorded behavioural observations during the morning hours into a log book on a daily basis. Keepers were asked to call in on day one of the oestrus behaviour and if this continued on day two, the A.I. was planned for day four of the natural heat. The males were anaesthetised first for

semen collection with the catheter method (LUEDERS *et al.*, 2012) and electroejaculation, thereafter the females were immobilised to perform the A.I. During A.I. the cats were placed in sternal position, with the hind end elevated slightly and fixation by the scruff of the neck. The vagina was mechanically stimulated by a cotton swap or 1 ml syringe. These measures were to simulate the natural mating traits.

Results

Behavioural oestrus was observed more frequently from December to February in AGC and between February and May in PL compared to the rest of the year and included increased vocalisation, scent marking and activity of the female with sometimes distinct response of the male (showing similar symptoms). Inter-oestrus intervals were between 10 and 21 days in ACG and 21 - 30 days in the PL. Oestrus lasted 3 - 5 days in both species.

In the morning of day 4 after onset of heat symptoms, the male was immobilised and semen was collected via urethral catheterisation (LUEDERS *et al.*, 2012). Ejaculate parameters are shown in table 1. Afterwards, electroejaculation was performed to receive additional volume. Usually three to five sets of 10 - 20 stimuli of 1 - 4 V were sufficient. Each semen sample was diluted 1:1 with cell culture medium (Medium 199, Sigma-Aldrich, Steinheim, Germany) and stored at room temperature until insemination.

The female was immobilised once semen was obtained. Transrectal and/or transabdominal ultrasound were performed using an endolinear probe (7 - 12 MHz, Logiq e, General Electrics Healthcare GmbH, Solingen, Germany) attached to an extension made of PVC pipe (1 cm diameter). One or two ovulatory follicles could be visualised in the females (table 1). When large, tertiary follicles were confirmed, the cat was put into sternal position and elevating the hind end. In this position, a commercial tomcat urethral catheter with stylette (Buster sterile cat catheter, WDT, Garbsen, Germany; 1.3 x 160 mm) could be passed through the vagina and cervical canal under transrectal digital control. Correct position was verified by transrectal ultrasound. The UC semen was inseminated into the uterine body, while the EE semen was placed deep into the vagina. An IM injection of the GnRH analogon buserelin (Receptal[®] Intervet International GmbH, Unterschleißheim, Germany) was given and the vagina was stimulated to induce ovulation (table 1). The females remained in position for another 15 min, before animals were placed back into their enclosures and reversed.

Pregnancy was confirmed by faecal prostaglandin F_{2α} metabolite (PGF-M) detection. A significant rise in PGF-M was previously reported in other pregnant felid species (FINKENWIRTH *et al.*, 2010; DEHNHARD *et al.*, 2012), and was used in the cases reported here as indicator for pregnancy. Levels rose after day 45 - 50 from about 1 µg/g to 10 - 70 µg/g in the AGC and after day 60 from about 20 ng/g to 80 - 390 ng/g in the PL.

After uneventful pregnancy, the ACG gave birth twice after 84 days (litter 1: 1,1 cubs, litter 2: 1,0 cub; interbirth interval: 12 months). The pregnancy length in the PL was 100 days with a litter of 1,1 (table 1).

The first time mother AGC raised 1,0 cub, whereas 0,1 was successfully hand-raised. Unfortunately 1,0 cub from the first litter and 1,0 from the second litter were found dead after 60 and 11 days respectively, without any obvious *post mortem* finding. The PL raised both cubs successfully to the age of currently seven months.

Tab. 1: Basic reproductive parameter recorded for 0,1 Asiatic Golden cat and 0,1 Persian leopard as well as 2,0 Asiatic golden cats and 1,0 Persian leopard; *) 3 semen collections in two males;
¹⁾ 2 semen collections in one male.

Parameter:	Species:	Asiatic golden cat	Persian leopard
Total ejaculate volume		60 - 280 µl*	120 - 900 µl ¹⁾
Total semen motility		70 – 85 %*	65 – 90 % ¹⁾
Sperm concentration		80 - 450 x 10 ⁶ / ml *	150 - 604 x 10 ⁶ / ml ¹⁾
Inter-oestrus interval		~ 10 - 21 days	~ 21 - 30 days
Oestrus duration		3 - 5 days	~ 3 - 5 days
Total uterine horn thickness in oestrus		0.7 cm	0.55 cm
Thickness endometrium in oestrus		0.45 - 0.51 cm	0.41 cm
Ovulatory follicle number/ diameter		1-2/ 0.45 - 0.51 cm	2/ 0.85 cm
Ovulation induction with Receptal [®]		0.75 ml IM	2.5 ml IM
Pregnancy duration (from day of AI till birth)		84 days	100 days

Discussion

To our knowledge, this is the first successful A.I. in Asiatic golden cats and the first time that Persian leopard cubs were raised after A.I. There is one report on a successful A.I. in one PL, the cub was born dead however (DRESSER *et al.*, 1982).

Details of the here reported A.I. technique were previously reported for the AGC (LUEDERS *et al.*, 2014). The same technical approach was now also successful in a Persian leopard. Although we have only performed a limited number of A.I.s, the fact that it worked repeatedly in the same ACG and at first trial in the PL, gives hope that this approach may be applicable to other feline species.

A combination of factors might have contributed to the immediate success of the A.I.:

1. Only one anaesthesia during natural heat (minimising stressful interventions);
2. Ovulation induction during anaesthesia (no previous darting with hormones, which may disturb or delay ovulation);
3. Fresh spermatozoa in high concentration collected via urethral catheter;
4. Manual stimulation of vagina, neck region in the females (simulating natural mating);
5. Sternal position during A.I., which facilitates passage of the cervical canal with the catheter and ensured intra-uterine placement of semen.

Acknowledgement

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DETECTION OF *CHLAMYDIA PNEUMONIAE* IN A SWISS SNAKE COLLECTION AND TREATMENT WITH MARBOFLOXACIN

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After a case of fatal infection with *Chlamydia pneumoniae* in a horned snake (*Vipera ammodytes ammodytes*) the presence of this pathogen was investigated in a private collection of 53 snakes comprising primarily European vipers, and also *Colubridae*, *Pythonidae* and *Elapidae*. In an exploratory screening six further animals were found positive for *C. pneumoniae* by real-time quantitative PCR (qPCR) and a subsequent ArrayTube microarray on swabs collected from choana and cloaca. Consequently, the whole collection was tested and, including the fatal case, *C. pneumoniae* was found in 8/53 (15.1 %) animals and an unidentified member of the *Chlamydiaceae*-family in one further animal. A treatment with marbofloxacin (Marbocyl FD, Vétoquinol AG, Ittigen, Switzerland) was implemented to attempt the eradication of *Chlamydia* from the collection. Four months after the first testing, all the positive animals ($n = 9$) and their cage mates ($n = 1$) were re-tested with qPCR-microarray before treatment. While three animals had become *Chlamydia*-negative, one of the cage mates that previously tested negative, had become positive. The seven positive snakes were treated with 5 mg/kg marbofloxacin IM SID, and tested for chlamydial infections (choana and cloacal swab) at the onset of therapy and every 5 days until day 35. The treatment was discontinued either as soon as all snakes in the same terrarium became qPCR negative - or no later than on day 35 for all the other snakes, independently from their microbiologic status, to allow clearance of potentially toxic antibiotic metabolites before the hypobiosis in hibernation which was scheduled to start on day 95. After hibernation (day 320), the complete collection ($n = 46$) was re-tested by qPCR-microarray to assess if the infection was still present. Four of seven animals were negative for *C. pneumoniae* after 10 days of treatment, while one became negative after 30 days. Two animals remained positive for *Chlamydiaceae* after hibernation, and one was sold and thus lost to follow-up.

In conclusion, this suggests that treatment with marbofloxacin might be efficacious against *C. pneumoniae* in snakes, although not entirely. Furthermore, molecular detection of *C. pneumoniae* was associated mostly with subclinical infections, suggesting that might not always be a primary pathogen in snakes. Finally, observed spontaneous elimination of the pathogen implies potentially effective immune response in snakes.

FLOW CYTOMETRY ANALYSIS OF PERIPHERAL BLOOD LEUKOCYTE POPULATIONS OF THREE BRAZILIAN SNAKE SPECIES: *BOA CONSTRICTOR*, *CROTALUS DURISSUS* AND *BOTHROPS JARARACA*

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Inflammatory processes are known to protect vertebrates from injuries and infections. However, from an immunological perspective the role of granulocytic and mononuclear leukocytes in snakes' inflammatory process is poorly understood. Within this context, leukocyte classification in these animals is not yet clearly defined, with authors disagreeing on existent cell types and their classification. Published data on reptilian leukocytes classification differentiates them into five cell groups: lymphocytes, azurophils, heterophils, basophils and monocytes. Nonetheless, the presence of eosinophils in the peripheral blood of snakes is occasionally described; their existence has not been proved in many species, except in *Ophiophagus hannah* and *Echis carinatus sochureki*. Azurophils are another leukocyte type in regard to classification. Some authors believe these cells are merely immature monocytes, while others consider azurophils to be cells with unique characteristics and roles, present only in reptiles with lesser numbers in lizards, chelonians and crocodylians, but greater numbers in snakes. Due to the great variation of snakes' leukocytes on cytochemistry, an analysis focused exclusively on optical morphology is insufficient to determine different cell types. Thus, additional studies and methods, as flow cytometry, are important to better understand the function and origin of each cell type. The objective of this research was to adapt leukocyte density gradients for snake blood samples, and characterise recovered cells based on size, presence of granules and internal complexity (organelle) by flow cytometry, enabling the qualitative and semi-quantitative assessment of cells constituting the immune system of *Boa constrictor*, *Bothrops jararaca* and *Crotalus durissus*. Blood samples were collected in tubes containing sodium heparin from 30 snakes (ten of each species: five males, five females). Thus, the fresh blood was centrifuged in the presence of either two commercial density gradients: Ficoll-Paque PLUS[®] and Percoll[®] (both GE Healthcare Life Sciences, Uppsala, Sweden). After centrifugation, leukocytes were recovered and analysed according to size and internal complexity by flow cytometry. Comparison between gradient methods for leukocyte isolation did not show any statistical difference (Kruskal-Wallis-Dunns, $P > 0.05$). Flow cytograms did not evidence differences in the distribution of populations between snakes of the same species, especially for mononuclear cells (skewness and kurtosis statistical test). When verified by means of flow cytometric cell sorting and confirmed by optical microscopy, populations were mainly composed of small lymphocytes, large lymphocytes, azurophils and heterophils. Our study identified cell populations and established leukocyte cytograms for *B. constrictor*, *B. jararaca* and *C. durissus* allowing functional and diagnostic assays through flow cytometry.

SEROPREVALENCE OF EQUINE HERPESVIRUSES TYPE 1 AND 9 (EHV-1 AND EHV-9) ANTIBODIES AMONG CAPTIVE AND WILDLIFE ANIMALS USING SPECIFIC PEPTIDE-BASED ELISA

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In *Equidae*, nine herpesviruses have been identified to date. Six of them belong to the subfamily *Alpha-herpesvirinae* and three to the subfamily *Gammaherpesvirinae*. Equine herpesvirus type 1 (EHV-1) and EHV-9 are associated with respiratory disorders and abortion in equids as well as neurological manifestations and death in equids and unnatural hosts such as polar bears. However, the host range for EHV-1 and the definitive reservoir for EHV-9 remain unknown. A novel sensitive and specific diagnostic tool, a specific peptide-based ELISA using two sets of peptides, 18 amino acids of EHV-1-glycoprotein E (gE) and 15 amino acids of EHV-9-gG, were applied to multiple species of captive and wild animals to determine the seroprevalence of EHV-1 and EHV-9. The EHV-1-gE peptide was used for discrimination between EHV-1 and EHV-4 as described previously. EHV-9-gG peptide has a 37 % similarity with EHV-1-gG and was used to differentiate between EHV-9 and EHV-1. To test the reactivity and specificity of the selected EHV-9-gG peptide, EHV-9 and EHV-1-positive and negative sera controls were used. The peptide produced high OD values (1.26) with an EHV-9-positive control and no reaction with EHV-1-positive (OD = 0.06) or EHV-9-negative (OD = 0.04) serum controls (Mann Whitney test, $P = 0.002$). Twelve families (*Equidae*, *Rhinocerotidae*, *Bovidae*, *Giraffidae*, *Cervidae*, *Hippopotamidae*, *Ursidae*, *Felidae*, *Canidae*, *Hyaenidae*, *Elephantidae*, *Cercopithecidae*) of animals from five orders represented by 428 serum samples collected from different zoos and wildlife were tested. Individual samples from the *Equidae*, *Rhinocerotidae* and *Bovidae*, were serologically positive for EHV-1 and EHV-9. The prevalence of EHV-1 was significantly higher in wildlife than in zoo zebras suggesting captivity may reduce overall viral exposure rates. EHV-1 infection was far more prevalent in zebras than EHV-9. Unexpectedly, EHV-9-prevalence was significantly high in rhinoceroses both in captivity and the wild, which may indicate they are an EHV-9 reservoir species. EHV-1 and EHV-9 have a broad host range among African mammals including distantly related perissodactyls, which should be considered in captive management of animals from Africa.

HEALTH STATUS OF GREY SQUIRREL POPULATIONS (*SCIURUS CAROLINENSIS*) IN PIEDMONT: ANATOMO-PATHOLOGICAL AND MICROBIOLOGICAL INVESTIGATIONS

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Summary

Forty grey squirrels of the province of Cuneo (Piedmont Region, north-west Italy) have been investigated with histological, microbiological and biomolecular techniques to increase the knowledge of the viral, bacterial, parasitic and fungal diseases affecting a grey squirrel population of Italy.

Histological lesions were found in the lungs, heart and skin. All the squirrels resulted negative for viral, bacteriological and parasitological analyses, except for the presence of bacteraemia in three squirrels. Mycological and biomolecular investigations revealed 14 squirrels positive to keratinophilic fungi belonging to nine different genera.

Introduction

The grey squirrel (*Sciurus carolinensis*), an American species introduced in Europe, is considered a pest with high potential for widespread, and represents a serious threat to the red squirrel (*Sciurus vulgaris*) because of their interspecific competition (MARTINOLI *et al.*, 2010). Since 2010 the northern regions of Italy (Liguria, Lombardy and Piedmont) have joined the LIFE + EC-SQUARE European Project, acting for the protection of the red squirrel through the control of the grey squirrel (BERTOLINO *et al.*, 2012).

The aims of this study were to increase the knowledge of the viral, bacterial, parasitic and fungal diseases affecting a grey squirrel population captured following a containment programme, and to assess possible risks to public health and for the transmission of pathogens to the red squirrel. *Post mortem*, histopathological, microbiological, parasitological and mycological investigations were performed.

Material and methods

Forty grey squirrels (21 males and 19 females) were captured and euthanised in 2013 and 2014 in the province of Cuneo (Piedmont Region, north-west Italy). Each squirrel was recorded with place and date of capture, the main biometric measures were registered, samples of the body surface were collected to detect dermatophytic fungi, and age was determined by the dry weight of the crystalline.

At necropsy, samples of eyes, heart, blood, skin, brain, faeces, liver, lacrimal and salivary glands, intestine, pleural fluid, central nervous system, spleen, skeletal muscle, ear, long bone, eyelid, hair, lung, kidney, stomach, uterus, testis and bladder were collected and fixed in 10 % buffered formalin (pH7) for histological investigations, and frozen at -20°C for microbiological and biomolecular investigations. Fixed tissues were routinely processed and stained with haematoxylin-eosin. Additional stains (PAS and Grocott for skin samples, and Alcian PAS and Weigert van Gieson for heart samples) were performed. Investigations for Hepatitis E virus, *Francisella* spp., *Salmonella* spp., Squirrel Poxvirus, *Toxoplasma gondii* and dermatophytic fungi were also carried out. All data were statistically analysed with the software GraphPad InStat (vers. 3:05; GraphPad Software, California, USA).

Results and discussion

Lesions were found in the lungs ($n = 28$: 25 BALT activation; 5 lymphocytic perivascular infiltration; 3 lymphocytic parenchymal infiltration; 3 hyperaemia and 1 macrophagic bronchitis), heart ($n = 28$: 25 endocardiosis of bicuspid valve; 9 valvular fibrosis of bicuspid valve; 5 endocardiosis of aortic valve; 2 endocardiosis of tricuspid valve; 2 myocardial fibrosis; 1 aortic fibrosis; 1 intramural artery hyalinosis; 1 liponecrosis and 1 myocardial malformation) and skin ($n = 10$: 8 lymphocytic infiltration; 2 scabs and 2 neutrophilic infiltration). All the squirrels resulted negative for viral, bacteriological and parasitological analyses, except for the presence of bacteraemia in three squirrels showing positive for *Staphylococcus* spp. and *Streptococcus* spp. Mycological and biomolecular investigations revealed 14 squirrels positive to keratinophilic fungi belonging to nine different genera (*Chrysosporium* spp, *C. keratinophilum*, *C. lobatum*, *Trichophyton terrestre*, *T. thuringiense*, *T. ajelloi*, *Arthroderma umbrium*, *Auxarthron conjugatum* and *Microsporum cookei*).

In Europe the main cause of extinction for the red squirrel is the competition for food with the grey squirrel; the competition is also mediated in UK and Ireland by a squirrel poxvirus, which so far is not found in Italy. However, other bacterial or viral pneumonia, fungal infections or parasites might be involved in the competition and should be investigated in the two species. Moreover, the role of the grey squirrel as a zoonotic carrier for mycotic diseases should not be underestimated.

Acknowledgement

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BRUCELLOSIS AND STORMY ABORTION IN PERSIAN GOITERED GAZELLE (*GAZELLA SUBGUTTUROSA SUBGUTTUROSA*)

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Summary

Around 35 years ago a number of Persian goitered gazelles (*Gazella subgutturosa subgutturosa*) were transferred from southern Iranian provinces to Kish Island, Persian Gulf. A number of them had entered the Kish Island's airport fences. Afterwards the fences were repaired, so the gazelles were trapped in there and could not get out for two years. After these two years, at the breeding season there were lots of fawns inside the fence and no fawn was found on the outside. Later there was a report of two female gazelles with haemorrhagic discharge from the vagina, outside fences. After all, researches revealed that in the past year many livestock which were imported illegally to the island, had abortions in the area. So, blood and serum samples were collected from livestock to do the Rose Bengal Test (RBT) and Complement Fixation Test (CFT) test. With positive test results and high antibody titers against *B. melitensis* in the tested livestock, efforts were made to capture the gazelles of the island. Sampling took place from nine gazelles outside the fence and eight gazelles inside. Sera were tested for antibodies to *Brucella* species by means of RBT and CFT. In the capturing process, one recently aborted foetus was also discovered and its amniotic fluid was used for *B. melitensis* culturing. Nine of ten livestock, nine of nine gazelles outside airport fence and zero of eight gazelles inside airport fence were considered positive based on reactions in the RBT and CFT. Amniotic fluid culture from the aborted foetus confirmed the existence of *B. melitensis*. This was the explanation for breeding in those gazelles which were surrounded in the fences and had no contact with the livestock and other gazelles outside.

Introduction

The goitered or black-tailed (*Gazella subgutturosa*) is a gazelle found in northern Azerbaijan, parts of Iran, parts of Iraq and south western Pakistan, south eastern Turkey, Afghanistan and the Gobi Desert. The specific name, meaning "full below the throat", refers to the male having an enlargement of the neck and throat during the mating season. It is considered vulnerable due to "IUCN red list of threatened species" since 2006. Persian goitered gazelle (*Gazella subgutturosa subgutturosa*) is a subspecies that is found in south eastern Turkey, Azerbaijan, Syria, northern and eastern Iraq, Iran, southern Afghanistan, and western Pakistan (ZIAIE, 2008) (figure 1 and 2).

Brucellosis is a highly contagious and important zoonotic disease caused by different species of the genus *Brucella*, small, Gram negative, non-motile, non-spore forming, rod shaped (coccobacilli) bacteria (BAEK *et al.*, 2003; KAKOMA *et al.*, 2003) that are pathogenic for a wide variety of animals and

also for humans (MATHUR, 1971). Basing on pathogenicity and host preference, six bacteria species are recognised, *Brucella abortus*, *B. melitensis*, *B. suis*, *B. ovis*, *B. canis* and *B. neotomae* (ALTON *et al.*, 1988). However, the principal pathogenic species worldwide are *Brucella abortus* (bovine brucellosis), *B. melitensis* (ovine and caprine brucellosis), and *B. suis* (swine brucellosis). *Brucella* spp. are facultative intracellular parasites causing chronic disease which may persist for the whole life of the affected organism. In animals, brucellosis mainly affects reproduction and fertility, reduces the survival of new-borns, and diminishes milk yield. The mortality of adult animals is insignificant (SEWEL and BLOCKLESBY, 1990).



Fig. 1: Persian gazelle (*Gazella subgutturosa subgutturosa*). (Photo: Shahrdari A)

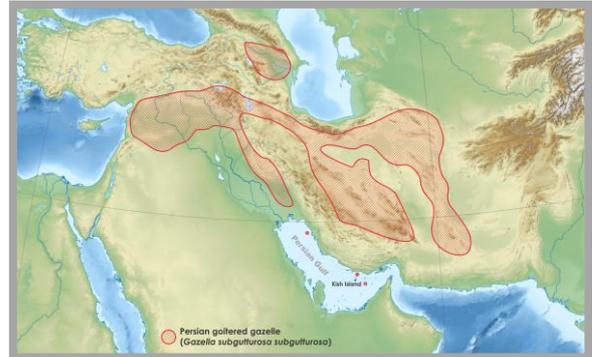


Fig. 2: Distribution of the Persian gazelle (*Gazella subgutturosa subgutturosa*) depicted as red-hatched area in the topographic map of south-western Asia. (Image: Jannati E)

Material and methods

Around 35 years ago a number of Persian goitered gazelles were transferred from southern Iranian provinces to Kish Island, Persian Gulf. Recently, there was a report of some gazelles entering the Kish Island's airport fences. They were able to enter that area because soldiers who wanted to escape the military service, had torn the fences. Afterwards the fences were repaired, so the gazelles were trapped in there and could not get out for two years. In spite of the efforts of the authorities, it had been two years that they were staying in that area and they had even reproduced. By the time authors efforts started to translocate the gazelles to free ranging areas, it was revealed that in breeding season there were lots of fawns inside the fence and no fawn was found on the outside. Rangers had reported two female gazelles with haemorrhagic discharge which they thought were from the anus, but later, after reviewing the photos, it was revealed that the discharges were from the vagina (figure 3). As the first step, these findings were the beginning of further researches on the domestic livestock. It was revealed that in past three years, the Veterinary Organisation had some plans to expel the livestock from the island. So by buying the livestock three times more expensive than the original price, it had discharged all those livestock. Afterwards the local inhabitants who had received a great amount of money, started to illegally import livestock, which had not been under vaccination programmes of the Veterinary Organisation. In later interviews with local herders, it was revealed that in the past year, many livestock had abortions and only two successful births had made place among 40 livestock (35 domestic sheep, five domestic goats) in the area. However external and internal parasitic infections and poor management systems were also obvious. With respect to the history, blood and serum samples were collected from nine livestock which had abortions and one which had healthy parturition, to do the RBT and CFT test. With positive test results and high antibody titres against *B. melitensis* in the tested livestock we started to catch the gazelles of the island by free darting and

drop net (figure 4). During free darting, anaesthesia was achieved using a combination of Ketamin 10 % (Alfasan, Woerden, The Netherlands) 4 mg/kg, Medetomidine HCL 20 mg/ml (Kyron Laboratories (Pety) Ltd., Johannesburg, South Africa) 70 µg/kg and Butorphanol 50 mg/ml (Kyron Laboratories) 0.4 mg/kg administered IM in a 1.5 ml dart by Daninject darting gun. This provided effective anaesthesia for the capturing process. Sampling took place from five female and four male gazelles outside the fence and two females and six males inside. During anaesthesia, animals exhibited hyperthermia due to the high temperature (more than 50°C) which was treated by fluid therapy and corticosteroids (figure 5). The anaesthesia was reversed with Atipamezol 5 mg/ml (Alzane Vet, syva s.a.u. León, Espanja/Spain) 0.35 mg/kg IV at least 20 min after Medetomidine injection.



Fig. 3: Haemorrhagic discharge from vagina, which could be the result of abortion or septic metritis after abortion. (Photo: Khaleghi Hamidi AH)



Fig. 4: Just at the time of capturing with drop nets, the animals were restrained physically to take the sample, reduce the stress level and avoid trauma. (Photo: Khaleghi Hamidi AH)



Fig. 5: Fluid therapy to reduce hyperthermia during anaesthesia. (Photo: Khaleghi Hamidi AH)

Sera were tested for antibodies to Brucella species (*B. abortus*, *B. melitensis* and *Brucella suis*) by means of the Rose Bengal Test (RBT) and the Complement Fixation Test (CFT). RBT was performed according to the procedure described by the OIE (OIE, 2008). The test serum samples and Rose-Bengal antigen (Razi Institute, Karaj, Iran) were kept for one hour at room temperature before the

beginning of the test. A result was considered positive when there was any degree of agglutination noticeable and the absence of agglutination was considered as negative.

For the CFT test, preparation of the diluents (Veronal buffer/CFT buffer), sensitised sheep red blood cells (SRBC), approved smooth *Brucella* antigen (Razi Institute), complement and sera was done according to the OIE Manual (OIE, 2004). Diluted test sera and working standards were placed in small tubes and incubated at 56°C for 30 min to inactivate the native complement. The CFT test was carried out in standard microtitre plates with round bottoms.

Volumes of 25 µl of diluted inactivated serum were added to the first, second and third rows. The first row was designated as an anti-complimentary control for each test serum. Diluent (25 µl) was placed in the first row wells to make up for the lack of antigen. Another 25 µl of diluent was added to all wells, but those in the second row. Serial two-fold dilutions were carried out and 25 µl was discarded at the end. Volumes of 25 µl of *Brucella* antigen were added to all wells, but those in the first row. Complement (25 µl) was then added to each well. Control wells with diluents only, complement and diluents, complement, diluents and antigen were set up. The plates were incubated at 37°C for 30 min, 25 µl of sensitised SRBC was added to all wells including control wells and re-incubated at 37°C for 30 min after thorough mixing by agitation. Results were read after the plates were left to stand for 1 h to allow unlysed cells to settle. Titres of 1:8 and above were recorded as positive based on the presence or absence of haemolysis.

Both tests were performed essentially as for bovines as described by Alton and co-workers (ALTON *et al.*, 1988) as no other specific tests have been validated for use in wildlife (CONDY and VICKERS, 1972; MADSEN and ANDERSON, 1995).

In the capturing process, one recently aborted foetus was also discovered and its amniotic fluid was used for *B. melitensis* culturing (figure 6). Even though one case is not sufficient for confirming the disease, but since finding and getting samples is very difficult in the wildlife field, using this one sample was beneficial. Serum dextrose agar (SDA – homemade medium) was used for culture of specimen. The plate was incubated for 10 days at 37°C in an atmosphere with 5 – 10 % CO₂ added. In parallel, the specimen was cultured in similar condition in an enriched liquid medium (serum dextrose broth supplemented with antibiotic mixture) for up to six weeks, with weekly subculture on to a solid selective medium (Farrell's homemade medium).



Fig. 6: A recently aborted foetus which was discovered during capturing process. It's amniotic fluid was used for B. melitensis culturing. (Photo: Shahrdari A)

Results

We investigated the presence of antibodies to *Brucella* spp. in sheep, goat and gazelles. The study showed that seropositive animals were present in livestock (90 %) and gazelles outside the airport fence (100 %), but not in those inside.

Nine of ten livestock, nine of nine gazelles outside airport fence and zero of eight gazelles inside airport fence were considered positive based on reactions in the RBT and CFT. All the RBT positive serum samples showed the titres of 1:8 and above in CFT test and they were recorded as positive.

Also eight gazelles (six males and two females) were captured inside the fence and their serum was used to do the RBT and CFT test. The results of these tests were negative for all of them.

Colonies typical for *Brucella* were checked with anti-*Brucella* standard serum, examined in catalase and oxidase tests and stained by Gram's Method. Further characteristics were determined by using monospecific anti-A and anti-M sera (AHVLA, Weybridge, UK) and further tests for CO₂ requirement, production of H₂S (Hydrogen Sulfide Test Strip, Fluka, WGK Germany) and urease, growth in the presence of thionin and basic fuchsin, and lysis by phages (Tbilisi at its routine test dilution – RTD and 104 × RTD). After all amniotic fluid culture from the aborted foetus confirmed the existence of *B. melitensis*.

Discussion

Brucellosis is an important zoonosis and serological surveillance is essential to its control (ERDENEBAATAR *et al.*, 2004). Although many countries have eradication programmes for controlling brucellosis, economic losses can be heavy due to abortion, infertility and subsequent culling so herds should be monitored for the presence of infection. Despite the eradication programmes, including vaccination, test and slaughter, brucellosis remains a major zoonosis worldwide (MATYAS and FUJIKURA, 1984; WHO, 1986; BAEK *et al.*, 2003; KAKOMA *et al.*, 2003) and the disease has remained prevalent in many areas in the world. Each year, half a million cases of brucellosis are reported worldwide but according to WHO, these numbers are greatly underestimated. In recent years, many countries have eradicated brucellosis from their herds, and many other countries have significantly reduced the prevalence of the infection among their livestock populations. Even so, brucellosis is distributed throughout the world wherever livestock are being raised. Likewise, in many less developed countries and in developing countries, brucellosis continues to cause major losses in livestock and poses a serious threat to people (CRAWFORD *et al.*, 1990). The distribution of the disease is geographically limited, but it nevertheless remains a major problem in Western and southern Asia, parts of Africa and Latin America (AMATO, 1995).

Iran Veterinary Organisation also has eradication programmes such as vaccination and test and slaughter, but they were not applied on the livestock in the island, because they had been imported illegally despite the efforts to expel all the livestock from the island.

The high proportion of seropositive gazelles in the island may similarly be attributed to contact with domestic livestock. Obviously, any plan to control brucellosis in domestic animals must take into account the sharing of grazing and watering areas with different kind of ruminants such as gazelles. Brucellosis is most likely to be the next disease for obligatory control in Iran and the increasing populations of gazelles in areas where there is increasing use of semiarid land for agriculture means that wildlife must be considered significant in any control measures developed for livestock.

Antibodies were detected in all ages and both sexes. Hygroma and orchitis were not observed in any of the seropositive animals. There existed a significant association in goats and sheep between abortion and the prevalence of brucellosis, seroprevalence in all the five sheep that had abortions was positive and it was negative in that one sheep which did not abort. Based on this, it can be concluded that the prevalence of brucellosis was higher in animals with a previous abortion record than in animals with no abortion record.

Seroprevalence is very subjective because it cannot distinguish between current and previous exposure to the organisms, therefore, there was a need to isolate and characterise the micro-

organisms. This helps to understand its epidemiology and devise proper control measures. Because the bacterium has a zoonotic potential, it is important to create public awareness to communities adjacent to the protected areas (FYUMAGWA *et al.*, 2009).

The negative results of the tests in gazelles which were captured and tested inside the fence, was the explanation for breeding among them. Since in the past two years that the fence was repaired, they were surrounded in that area and had no contact with the livestock or other gazelles outside.

The high percentage of seropositive animals in the island must have been due to importing livestock illegally and that it was the first time that these gazelles were exposed to brucellosis. Otherwise such stormy abortions would not have happened.

The plan for decreasing the infection among these animals could be transferring all the livestock away from the island, or putting them under eradication programmes of the Veterinary Organisation. We should also keep on monitoring the gazelles to see if the abortion rate decreases in the next breeding season.

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THE PLIGHT OF THE NORTHERN WHITE RHINOCEROS (*CERATOTHERIUM SIMUM COTTONI*): IS THERE STILL HOPE TO PREVENT EXTINCTION?

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With only five surviving individuals and the prospects for reproduction compromised, the northern white rhinoceros (*Ceratotherium simum cottoni*, NWR) has been considered to be a form of rhinoceros doomed to extinction. It may serve as a notable example in the first years of the new century of a species for which the only hope for survival is the further development and application of advanced reproductive and genetic technologies. The related southern white rhinoceros (*Ceratotherium simum simum*) is relatively numerous and viable hybrids have been produced with NWR. Semen has been saved from several NWR in Berlin and San Diego. Furthermore, the Frozen Zoo[®] of the San Diego Institute for Conservation Research has viable cell cultures from 12 NWR, arguably a sufficient gene pool to preserve a species. Fibroblast cells from Fatu, the youngest female NWR, have been transduced to become induced pluripotent stem cells (iPSCs), capable of making any tissue in the body. These resources may form the basis for an ambitious effort, involving many steps, to employ advanced genetic and reproductive technologies to save the NWR. Initial efforts in San Diego are focused on comparing whole genomes of northern and southern white rhinoceros, further stem cell work, and the establishment of a cohort of reproductively capable female southern white rhinoceros to serve as ovum donors and surrogates for gestation. The genome data will shed light on the extent of genetic variation surviving in both types of white rhinoceroses, suggest their demographic histories, and allow a quantitative assessment of their genetic divergence gene by gene. These data will contribute to resolving their status as species or subspecies. The stem cell efforts will strive to produce a full representation of the surviving gene pool as represented by the living animals and the cells in the Frozen Zoo[®]. Further efforts will seek to produce gametes from IPS cells as has been done in the mouse. Non-surgical ovum pick-up (OPU) has been undertaken, in vitro fertilisation and intracytoplasmic sperm injection have been attempted performed in southern white and black rhinoceroses, but further efforts are required for achieving embryogenesis. Besides the successful application of transcervical artificial insemination in white rhinoceroses suitable techniques for embryo transfer have to be developed for this rhinoceros species, as it has been done in other non-domestic species. These various steps, while a speculative venture, utilise approaches that have been successfully developed and applied in other species. Furthermore, they represent the best hope for avoiding the extinction of the NWR. In the end, efforts to develop these technologies for genetic rescue will assist in efforts for other species in the future.

MORTALITY OF WILD SRI LANKAN ELEPHANTS (*ELEPHAS MAXIMUS MAXIMUS*) AS A RESULT OF *PARABRONEMA SMITHI* INFESTATION

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The Sri Lankan elephant (*Elephas maximus maximus*) is considered as a unique sub species of Asian elephants. In IUCN red list its conservation status is endangered. There are around 6,000 wild elephants living in Sri Lanka that represent more than 10 % of Asian elephants in the world. On average 200 deaths of wild elephants per year are recorded in Sri Lanka. The majority of elephants die due to human intervention and less than 10 % of deaths are considered due to natural causes. The old age is considered as the most common natural cause for the death of wild elephants but the results from this study reveals otherwise. Therefore this study was conducted to reveal the parasite-host relationship of *Parabronema smithi* as a cause of wild elephants irrespective of their age.

It is mandatory to conduct a *post mortem* when a wild elephant dies to verify and record the cause of death. In most instances carcasses of wild elephants with suspected cause of death as 'natural' are decomposed. Therefore, in many cases it is difficult to conduct a detailed *post mortem* examination to reveal the exact cause of death. Over a two year period since 2012, eleven fresh elephant carcasses have been encountered in the southern wildlife region in Sri Lanka suspected of death due to natural causes. The *post mortem* of all 11 carcasses was conducted within 24 h of death. Seven of these animals have died while the main author was treating them as veterinary intervention on wounded and sick wild elephants are common practice in Sri Lanka. Among these 11 deaths, there were intensively spread caseous ulcers in the stomach wall in nine animals. It was the only gross suspected lesion of eight of them. The size of those ulcers varied between 0.4 - 8 cm. The margins of ulcers were elevated and the tiny parasites were observed in the ulcers as well as nearby mucosa of the inner stomach wall. Of the nine animals, three were male and six were females. The ages of the males were 2, 6 and 14 years. The females were older: four were over 30 years and two of them about 20 years old. Four of these females were also lactating. All the infected animals were emaciated. Using morphology, the parasite species found in the stomach was identified as *Parabronema smithi*. The average size of this parasite varies between the sexes (female 7.11 ± 0.5 mm; male 5 ± 3 mm). It belongs to Spiruridae family. Histopathological investigations revealed that areas of the gastric mucosa were lost and covered with hypereosinophilic cellular debris (necrosis) surrounded by a band of inflammatory cells predominantly composed of polymorph multinucleated cells, lymphocytes and plasma cells. There were multiple intralesional nematode cross sections as well in the gastric mucosa. Some of the parasite cross sections contain larvae in the uteri indicative of their adult stages.

Although this parasite has been listed as one of recorded parasite species in Sri Lanka, there is no relevant information of reported places or pathological changes. The results of this study revealed that *Parabronema smithi* infections have significant contributions on mortalities of elephants in the wild in Sri Lanka. This parasite may have been with wild elephants for a long time but due to some ecological factors such as stress associated with shrinking habitat, restrictions of elephant migratory pathways, increasing of elephant density per land area and other types of human interventions may be changing the parasite host relationship. Currently we are conducting further research to find out the prevalence of this parasite among wild elephants and pathological significance of them.

DISTRIBUTION AND LOAD OF ELEPHANT ENDOTHELIO TROPIC HERPESVIRUS DNA IN TISSUES FROM ASSOCIATED FATALITIES IN ASIAN ELEPHANTS (*ELEPHAS MAXIMUS*)

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Insight into the pathogenesis of elephant endotheliotropic herpesvirus (EEHV) infections can be carried out primarily by examining EEHV-infected tissues due to lack of an animal or *in vitro* cell model. Little is known about distribution and burden of the virus within the organs of fatally affected Asian elephants (*Elephas maximus*), which is crucial in understanding the virus pathogenesis.

In this study, the extent of organ tropism of EEHV in four fatal cases of EEHV-1A (total of 40 tissue samples), one fatal case of EEHV-1B (7 tissue samples), and one of EEHV-5 (9 tissues samples) was assessed using several quantitative real-time PCRs. Viral DNA for EEHV-1 and EEHV-5 were detectable in all the tested tissues (aorta, blood, heart, kidney, liver, lung, lymph nodes, spleen, thymus, and tongue) although with significant differences in viral DNA load. The highest EEHV-1A DNA load was observed in the liver, followed by heart, thymus, and tongue. EEHV-1B and EEHV-5 showed the highest DNA load in the heart, followed by tongue and liver. However, whether higher viral load in certain tissues originates from the presence of a more extensive vascular system or from the virus targeting other cells in these tissues is yet to be determined. Also, genetic variation between EEHV-1A, EEHV-1B, and EEHV-5 may also account for the differences in tissue tropism of the viruses.

This study provides new insights into EEHV pathogenicity and has implications in choice of sample type for virus isolation and disease investigation.

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PUBLIC AQUARIUM MEDICINE AND SURGERY – ADVANCES IN THE PAST 20 YEARS AND CHALLENGES AHEAD

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As little as 20 years ago, veterinary input to public aquaria was minimal with vets rarely setting foot in an aquarium and an aquarist attitude of “if it dies it can be replaced” and “vets do not know anything about fish” rife in the industry. At this time there was already a significant knowledge base in the veterinary profession surrounding farmed fish but there was little crossover to the public aquarium industry. The changes in the past 20 years have been enormous and this talk will describe these and discuss the current levels of medical and surgical interventions, quarantine procedures and prophylactics, diagnostics and facilities and equipment now standard in public aquaria.

The current levels of both veterinary medicine and veterinary pathology input into management of public aquaria have led to enhanced husbandry and welfare. The potential for further advance is huge but we are challenged by the increased global movement of fish leading to new pathogens being found in previously unaffected species. The industry is also constantly evolving and expanding and the expectation by the public to see ever more unusual species means that more difficult or sensitive species are being trialled. This often means that there is a steep learning curve for both the aquarists as well as the vets as we regularly have to deal with new species.

Members of EAZWV vary from full-time aquarium veterinarians to those who never go near a live fish. This talk has been prepared to try to encourage more zoo veterinarians to take an interest in what is going on in their aquarium. Encouraging more zoo veterinarians to get involved with fish can only be a good thing for the future development of techniques and practices in public aquaria.

ADDITIONAL ULTRASONOGRAPHIC FINDINGS IN THE FOURTH TRIMESTER OF PREGNANCY IN BOTTLENOSE DOLPHIN (*TURSIOPS TRUNCATUS*) UNDER HUMAN CARE

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The use of ultrasonography during the dolphin pregnancy has usually the following focuses: foetal movements, foetal heart rates, measurements of the skull and the thorax for the birth prediction, the morphological aspects of the organs, the appearance of the umbilical cord and the placentation. This study reports additional ultrasonographic findings. Portable Sonosite 180 plus (Esaote s.p.a. International Activities, Firenze, Italy) with convex probe 5-2-MHz, MyLab™25Gold (Esaote s.p.a. International Activities) with convex probe 3,5-MHz and Aloka SSD-900 (Aloka Co., Ltd, Tokyo, Japan) with convex probe 3,5-MHz were used to evaluate 12 females during pregnancy. Ultrasonographic examinations were performed every four days during the fourth trimester. In our experience, if the foetus has the tail next to the Corpus luteum, it will have a cephalic partum; instead, if it has the skull next to the Corpus luteum, it will have a podalic partum. In addition, we observe caudally to the bulbo-urethral muscles a hyperechoic area which can be related to the base of the penis. During the last 20 days of pregnancy, allantoic fluid increases in volume and shows an increase of the echogenicity compared with amniotic fluid. Statistical analysis of the data collected from 276 examinations shows that the amniotic fluid was more echogenic than the allantoic fluid ($P < 0.05$). This study expands the present knowledge for ultrasonography in bottlenose dolphins and provides additional relevant data to the clinician during dolphin pregnancy.

MYCOBACTERIUM PINNIPEDII / AFRICANUM IN SOUTH AMERICAN SEA LIONS (*OTARIA FLAVESCENS*) IN AALBORG ZOO

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In the period of 2009 to 2014, seven South American sea lions (*Otaria flavescens*) were euthanised or died in Aalborg Zoo. Three sea lions were wild caught animals from Uruguay. *Post mortem* findings of the first four cases were chronic granulomatous pneumonia and enlarged lymph nodes. Only in the fourth animal, acid-fast bacteria was found by microscopy.

Two remaining females were tested for mycobacteriosis by culture and microscopy of broncho-alveolar lavage at Statens Serum Institut (SSI) and The National Veterinary Institute (NVI) in 2012. Serum from both animals was tested at the Central Veterinary Institute from Wageningen UR (CVI). All samples were negative. The Zoo then decided to import a captive born male tested negative by PCR and serology for Mycobacteria.

In 2014, a female was euthanised due to chronic lameness. Lungs and lymph node were culture and PCR positive for *Mycobacterium pinnipedii / africanum* at both SSI and CVI. The sea lion enclosure was quarantined and all movements out of Aalborg Zoo were stopped. The two sea lions left had no clinical symptoms, but one died during anaesthesia and the other was euthanised half year later. Both were diagnosed with mycobacteriosis.

In retrospect, it appears likely that the first four necropsied animals were infected with *M. pinnipedii*, highlighting the protracted and often cryptic nature of this disease. These cases confirm the necessity of applying several and regularly repeated diagnostic tests of live South American sea lions (JURCZYNSKI *et al.*, 2012) which was not done with the initial cases in Aalborg Zoo.

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DEALING WITH AN OUTBREAK OF *MYCOBACTERIA PEREGRINUM* IN AUSTRALIAN LUNGFISH (*NEOCERATODUS FOSTERI*) USING LONG-TERM TRIPLE ANTIBIOTIC THERAPY

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A group of captive bred juvenile Australian lungfish (*Neoceratodus fosteri*) was imported from Australia to ZSL London Zoo. Members of this group developed skin lesions, demonstrating acid-fast bacteria on impression smears. *Mycobacterium peregrinum* was isolated from the lesions. Several fish died or were euthanised due to the severity of the lesions and disseminated internal mycobacteriosis due to *M. peregrinum* was confirmed *post mortem*. The remaining fish were isolated as a group, maintained in quarantine on a separate filtration system at 25.5 - 26.5°C and daily triple therapy of 10 mg/kg rifampicin (Rifadin[®], Sanofi-Aventis S.P.A., Anagni, Italy), 2 mg/kg doxycycline (Doxycycline, Actavis, Barnstaple, UK) and 10 mg/kg enrofloxacin (Baytril[®] Bayer plc, Newbury, UK) was initiated. Treatment was individually dosed orally, and accurate dosing was ensured by weighing the treated fish monthly. There was visible regression of skin lesions within the first month. By the second month of treatment, lesions had resolved and impression smears and cultures were negative, remaining so for the six months of follow-up investigations in both affected and non-affected in-contact fish. Eight months after starting treatment, treatment was discontinued. No adverse effects were noted to therapy and the fish grew normally. There has been no recurrence to date, five months after discontinuing treatment. Mycobacteriosis is often treated in mammals with long-term, triple-drug combination therapy to overcome the resistant nature of these organisms. However, it is not often treated in fish as accurate long-term therapy is difficult to ensure in an aquatic environment. This outbreak demonstrates that, in cases where full compliance and intensive treatment and monitoring can take place, triple therapy may be effective and appears to be well tolerated. This finding may be applicable to other valuable aquarium fish species.

A RETROSPECTIVE REVIEW OF PATHOLOGICAL FINDINGS IN ELASMOBRANCHS SUBMITTED TO INTERNATIONAL ZOO VETERINARY GROUP PATHOLOGY BY SEA LIFE CENTRES

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Retrospective histopathological (mainly *post mortem* surveillance, occasionally biopsy) findings are reported from elasmobranchs submitted to a specialist diagnostic laboratory by Merlin Entertainments Group's Sea Life attractions from May 2003 to June 2013. Cases included animals with varying clinical histories of illness as well as unexpected deaths without premonitory signs. Diagnoses were classified by pathological process and aetiology where possible. Submissions from 632 fish were received, across 72 different species. The most frequent were blacktip reef sharks (*Carcharhinus melanopterus*, 10.8 %), cow-nosed rays (*Rhinoptera bonasus*, 9.5 %), thornback rays (*Raja clavata*, 7.3 %), bonnet-head sharks (*Sphyrna tiburo*, 6.6 %), small-spotted catsharks (*Scyliorhinus canicula*, 6.0 %), zebra sharks (*Stegastoma fasciatum*, 5.4 %) and nursehounds (*Scyliorhinus stellaris*, 5.1 %). For all elasmobranchs across all years, the following pathological processes were identified: no pathological diagnosis (18.1 %), infectious and/ or inflammatory (41.0 %), nutritional (32.6 %), traumatic (2.4 %), suspected toxicosis (2.3 %), degenerative (1.9 %), deposition (0.8 %), reproductive (0.4 %), neoplastic (0.5 %) and environmental or mechanical plant failure (0.8 %). Aetiological agents identified included: bacterial infection (12.2 %), nematodes (7.5 %), trematodes (6.0 %), scuticociliates (4.6 %), coccidia (4.3 %), myxozoans (2.4 %), fungi (2.0 %), Epitheliocystis-like organisms (1.7 %), other protozoa (0.8 %) and suspected viral infection (0.5 %). These results supplement previous analyses of disease in aquarium elasmobranchs, and the evidence to improve husbandry and future disease investigations.

DECOMPRESSION SICKNESS IN SEA TURTLES: KEY DIAGNOSTIC AND THERAPEUTIC ASPECTS AND NEW PROGNOSTIC CONSIDERATIONS

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Decompression sickness (DCS) has been recently recognised as a new pathologic entity in by-caught marine turtles coming from trawling and gillnet fisheries of the Valencian Community region, Spain. The main goal of this presentation is to provide the veterinarians working with this species in rehabilitation centres, with some crucial diagnostic and therapeutic information to maximise post-release success of affected animals and to look for decompression compatible signs in dead ones. Systematic evaluation of DCS compatible signs in all by-caught animals received at our centre during the last 4 years ($n = 85$), revealed a relatively high prevalence of disease (48 %).

Diagnostic imaging has shown to be the most reliable approach for gas embolism confirmation in live and dead individuals. This includes relatively common and affordable veterinary techniques including plain radiography or basic ultrasonography. The most consistent diagnostic regions to explore for a quick evaluation of the patient are kidneys, heart and liver, showing different degree of intravascular gas accumulation depending on severity. Careful dissection of deceased individuals could permit the pathologists to directly visualise the presence of gas in certain key anatomical regions including right atrium, *sinus venosus*, mesenteric veins and postcava vein. However, in less severe cases it is rather complicate to visualise any gas on necropsy. Some necropsy findings in dead stranded sea turtles, though highly compatible with the condition, could not be fully confirmed to be consequence of DCS due to putrefaction interference. Other gross lesions commonly present in confirmed decompressed cases are congestive/haemorrhagic areas in the kidneys, liver and/or in the intestinal mucosa. On histology the main findings are represented by intravascular gas bubbles associated with general congestion and perivascular haemorrhages in a variety of tissues.

In our experience, disease outcome greatly depends on total amount and distribution of intravascular gas, time to hyperbaric oxygen therapy (HBOT), severity of clinical signs and evidence of pulmonary water aspiration. Adapted human diver's recompression-decompression tables have been progressively readjusted with time towards longer treatment regimes for turtles. The condition is generally lethal in moderate and severe cases not receiving HBOT, and could result in sequelae, even in mild cases. Kidney lesions were observed through magnetic resonance and computed tomography imaging in surviving individuals several days after gas reabsorption and blood profile normalisation, indicating a temporal and potentially permanent loss or renal functional capacity. Long term neurologic damage has been also associated with late HBOT treated mild embolism. Therefore, we consider all professionals dealing with bycaught or forcedly submerged sea turtles should be familiar with these diagnostic and therapeutic techniques in order to minimise sea turtle mortality or any other risks associated with organic damage in survivors.

AVIAN BORNAVIRUS – AN OVERLOOKED INFECTION IN EUROPEAN WATERFOWL

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Avian bornavirus (ABV) was first proposed as the cause of proventricular dilatation disease in psittacine birds in 2008 by two independent groups of researchers. A distinctly separate genotype (ABBV-1) was discovered in 2009 in free-ranging waterfowl in Ontario, Canada. A high prevalence (10 – 50 %) of this waterfowl-associated genotype has since been detected across North America in multiple avian species, mainly waterfowl. In 2014 a related strain was identified in mallards (ABBV-2), but none of the waterfowl genotypes have previously been detected in wild birds outside North America.

In order to investigate the presence of ABBV-1 in European waterfowl, the brains of 333 hunter killed geese (135 greylag goose (*Anser anser*), 103 pink-footed goose (*Anser brachyrhynchus*), 50 barnacle goose (*Branta leucopsis*), 16 white-fronted goose (*Anser albifrons*), 13 Canada goose (*Branta canadensis*), eight tundra bean goose (*Anser fabalis rossicus*), seven taiga bean goose (*Anser fabalis fabalis*) and one hybrid between greylag goose and Canada goose) from nine sites across Denmark were examined for ABBV-1 by real-time RT-PCR as previously described.

Five greylag, one pink-footed and one barnacle goose (2.1 %) were positive, and sequencing demonstrated 98.18 to 99.83 % identity among sequences, and 97.38 to 98.06 % similarity with the reference strain of ABBV-1 (Genbank accession number KF578398).

This is the first finding of ABBV-1 in wild waterfowl in Europe, and extends the range of waterfowl species in which the virus has been detected to include the pink-footed goose, greylag goose and barnacle goose. Given the migration pattern of these species, avian bornavirus is likely to have a much wider geographic range than has previously been suspected.

MAIN AND ADVANCED SURGERY IN AMPHIBIANS

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Literature is still rare on surgery in amphibians maybe due to the fact that the large number of species demand special knowledge of the anatomic and physiologic situations. Still, amphibians are generally good candidates for surgery. Most procedures described in reptile (mostly lizards) can be undertaken in most amphibians if equipment can be matched to patient size. The basic surgical concepts are: know the anatomy, control haemorrhage, if you do not know what it is, be gentle with it! Fasting these animals before surgery is usually not necessary unless a gastrointestinal procedure is planned. General anaesthesia and analgesia are required. If possible, animals are intubated. Heart rate and respiratory rate are useful tools for anaesthesia monitoring. Commonly performed surgical procedures include wound debridement and repair, abscess/neoplasm removal, tail/digit amputation, prolapse replacement/repair, endoscopy and coeliotomy for organ biopsy, reproductive sterilisation, or gastrotomy. In addition, ophthalmologic surgery will also be discussed here. One may use a combination of cold steel, radiosurgery or diode laser. Because the amphibian's skin is not very extensive, closure of large skin defects is sometimes impossible and second-intention healing is generally necessary. Monofilament nylon seems to be the most appropriate suture in amphibian skin. Haemostasis in mild haemorrhagic procedure may be achieved by electrocauter or diode laser. Laparotomy procedures follow the same technique as seen in lizards. The surgeon must take care of macroscopic glands, lymph hearts, and blood vessels, especially the midventral vein. Eyelid retractors can be useful for retracting coelomic incisions. In all cases, one will take care to prevent dehydration of all exteriorised organs and the skin during the procedures. No coagulation is needed for withdrawing eggs. Ovariectomy and orchidectomy may need mild electrocautery or the use of diode laser. Because the eyeballs can bulge out far and may sink deep, adequate eye position has to be obtained and maintained for the entire length of the surgery. Corneal incisions are made with a microsurgery knife. Globe dilation may be improved with intracameral injection of a viscoelastic substance. Cornea incisions are sutured applying simple interrupted sutures with 8 - 0 polyglactin suture material.

RETROSPECTIVE STUDY OF CRYPTOSPORIDIOSIS IN ZOO AND AVIARY BIRDS: 269 CASES

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Cryptosporidiosis has been occasionally documented in several non-domestic avian species (DA SILVA *et al.*, 2010; MOLINA-LÓPEZ *et al.*, 2010). A retrospective search of the pathology records of Noah's Path (2008 - 2014) and Northwest ZooPath (1995 - 2013) revealed 269 cases, particularly involving passerine (62.83 %) and psittacine (27.51 %) birds. Included are several endangered or vulnerable species such as the hyacinth macaw (*Anodorhynchus hyacinthinus*), red crowned crane (*Grus japonica*), red siskin (*Carduelis cucullata*) or tawny frogmouth (*Podargus strigoides*). In most birds (232/269), a single tissue target was identified, most commonly the proventriculus (191/269), followed by the intestine (27/269) and cloaca (14/269). Concurrent infections or parasitism were found in 163 birds. Of these, fungal infections were the most common (71/163), particularly macrorhabdiosis, followed by parasitic (52/163), bacterial (33/163) and viral (9/163) diseases. Cryptosporidia-like protozoa were associated with a variety of lesions in infected mucosae including mucous cell hyperplasia of proventricular mucosa, mucous metaplasia of proventricular glands, inflammation, and atrophy and fusion of intestinal villi, as well as with gastrointestinal bleeding and atrophy of adipose tissue; infection of epithelia without any associated lesions was also observed. This study suggests that cryptosporidiosis may be a common parasitic disease in zoo and aviary birds, particularly passerine and psittacine species, and can be frequently associated with concurrent infectious and parasitic diseases. The proventriculus appears to be the main target. In domestic birds, cryptosporidiosis may enhance the pathogenicity of some infectious agents (HAO *et al.*, 2008). The authors hypothesise that cryptosporidiosis may increase mortality in zoo birds, particularly when occurring in mixed infections or if associated with severe lesions of infected epithelia/mucosae.

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DOCUMENTATION OF GASTRIC PNEUMATOSIS LESIONS IN BLACK AND WHITE RUFFED LEMURS (*VARECIA VARIEGATA VARIEGATA*) WITH THE USE OF RADIOLOGY AND COMPUTED TOMOGRAPHY AND THE EFFECT OF DIET ON CLINICAL SIGNS

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Pneumocystoides intestinalis (PCI) is a disease more commonly described in humans and characterised by multilocular gas-filled cystic spaces located within the wall of the gastrointestinal tract. In humans these cystic spaces can occur in any location along the gastrointestinal tract as well as within the associated connective and lymphatic tissues. PCI lesions were identified in several black and white ruffed lemurs (*Varecia variegata variegata*) at the Tulsa Zoo and have been documented in another lemur species previously. Unlike the human disease PCI in these lemurs was associated with marked eosinophilic inflammation. In affected lemurs at the Tulsa Zoo clinical presentation has varied and included: death without premonitory signs, acute abdominal crisis from gastric intussusception and chronic vomiting. Vomiting has been an ongoing problem within the group and gastric pneumatosis could be seen in some of the animals' *ante mortem* via survey radiographs and an upper gastrointestinal contrast study. A clinical trial was undertaken to better document gastric PCI in this lemur group through survey radiographs and computed tomography (CT). Four of the eight lemurs received complete physical examinations, survey radiographs and CT scans of the thorax and abdomen with IV contrast media to assess the status of their stomach wall thickness and to screen for the presence of intramural pneumatosis lesions. The four lemurs were selected from the group as either known affected (had previously documented lesions via radiographs or surgery), suspected (observed vomiting) and one suspected unaffected (no observed vomiting) as a control. CT scan was better at identifying PCI lesions in the stomach wall than radiographs and all four lemurs including the 'unaffected control' were found to have gastric PCI lesions. The diet was modified to lower starches, and increase fiber and fat content to better match the nutrient profiles that published studies have shown are eaten by wild *Varecia* sp. This dietary change has been overall well accepted by the lemurs and the reported incidence of vomiting within the lemur troop has been significantly reduced. A repeat of complete physical exams, survey radiographs and CT scans are scheduled at approximately 12 months post diet change to assess if PCI lesions size and severity have been impacted by the new diet. No aetiology, such as a fungal infection or a parasitic infection, has been identified as the cause of the eosinophilic gastritis in these black and white lemurs.

3' 04 ENDODONTIC THERAPY ON A LEOPARD (*PANTHERA PARDUS*)

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Summary

During the first months of 2014, zookeepers reported a progressive weight loss in a six year old male leopard (*Panthera pardus*). In May 2014, it was diagnosed with fracture of four canines. The leopard was immobilised by blowpipe with a combination of ketamine and medetomidine in order to take X-rays and perform a thorough examination. Four canine teeth showed excessive wear with the pulp canal exposed. When the pulp canal was exposed by excessive wear the neurovascular bundle showed an infection and necrosis. A three root canal treatment and one crown filling were completed to eliminate pain.

Introduction

Endodontic therapy is used to treat the endodontic system formed by the root canal and the pulp cavity. The pulp is made up of blood and lymphatic vessels, nerves, odontoblasts, fibroblasts, and other undifferentiated mesenchymal cells. Carnivores' canines' essential function is to seize and hold prey. But this is not their only function; carnivores' canines are also used to first tear and later chew the prey's meat. Common causes of pulpar exposition found in canines are fracture or wear. Canines are teeth which allow predators to hunt and survive and these remain practically intact during carnivores' lifetime in the wild. Animals in captivity are fed by their keepers who decide nourishment form and size. In this case, we suspect that exposure of the pulp canal occurred because of excess wear, probably caused by the animal's habit to gnaw abrasive materials, such as the perimeter bars or stones and other structures in the leopard's enclosure.

Material and methods

A six year old leopard was observed to have lost weight at the Vigo Zoo in A Madroa. Observation of routine photographs taken showed that all four canines were fractured or badly worn, so anaesthesia was performed to evaluate the teeth condition. Clinical and radiologic examination showed pulpar exposition in three of the canines. The fourth canine was worn but the pulp was not exposed and there was no apical affectation. It was decided implementation of root canal therapy on the three canines with pulp exposition, and a dental filling to the fourth. The anaesthesia was induced with ketamine (ketamidol 100 mg/ml, Richter pharma ag, Wels, Austria) and medetomidine (Domtor 1 mg/ml, Esteve, Barcelona, Spain) 3 mg/kg plus 0.07 mg/kg. Local anaesthesia was performed with 2 ml Mevipacaine Normon[®] (Normon S.A, Tres Cantos, Madrid, Spain) 2 % on each mandibular and maxilar nerves. The pulp chamber was drilled to widen the root canal and the vasculous and nerval structure drilled out with a Hedgstrom file. In this case necrotic tissue was removed but the teeth were not extracted. Depth of the root canal was estimated thru X-ray and pathfinders to avoid damaging the apex when

using the H files. Internal widening of the root canal was performed with 55 H files. The endodontic access was washed with sodium hypochlorite and dried with paper points. Endodontic seal material Tubliseal EWT[®] was used as sealer with a 60 mm lentulo spiral and gutta-percha paper points of various diameters until complete filling of root canal. After removal of leftover fragments, the canal obturation was completed with glass ionomer cement (GIC).



Fig. 1: The leopard with teleinject dart 5 cc on shoulder area. (Photo: Acuña A)



Fig. 2: Leopard canines worn gingival level. The three canines with pulp canal exposed. Also some loose mandibular incisor teeth were detected. (Photo: Acuña A)

Results and discussion

Necrotic tissue that caused pain during mastication was removed and all four root canals were filled and sealed. Diagnosis of odontological problems in zoo animals is only possible if the animal is thoroughly examined under anaesthesia or, like in our case, if tooth wear is evident and there are clear eating disorders caused by odontologic problems. Removal of necrotic tissue and nerve, and disinfection eliminates pain on the area affected and therefore eases mastication of meat presented in smaller portions. Dental implant could be an adequate solution to restore canines' functionality to tear meat. However, since external agents contributing to the problem (such as the structural elements of the animal's enclosure) cannot be easily reformed, implant placement would not be useful on the long term as the leopard will eventually break them, or wear them out just as the animal had done with its own canines. This is the reason why removal of infectious process was the only procedure implemented along with a dietary protocol based on smaller meat portion intake.

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Fig. 3: Determining the depth of the pulp canal and extraction of neurovascular bundle with a brouch in maxilar canines. (Photo: Acuña A)



Fig. 4: Widening the pulp canal with 55 mm Hedstrom files. (Photo: Acuña A)



Fig. 5: Maxilar canines pulp canal widened, disinfected and dried. (Photo: Acuña A)



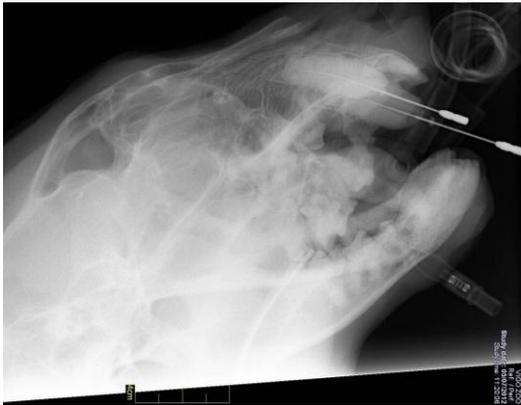
Fig. 6: Filled pulp canal with tubliseal and veterinary gutta-percha points. Pulp canal must be completely filled without leaving air spaces. (Photo: Acuña A)



Fig. 7: Mandibular teeth with different wear. (Photo: Acuña A)



Fig. 8: Crown seal with adhesive and composite. (Photo: Acuña A)



*Fig. 9: X-ray for confirmation of maxillary canines pulp canal depth.
(Photo: Acuña A)*



*Fig. 10: Checking depth of the mandibular canine teeth pulp canal and endodontic treatment in maxillary canine teeth by X-ray.
(Photo: Acuña A)*



Fig. 11: X-ray of endodontic treatment in the three canine teeth. (Photo: Acuña A)

TREATMENT OF BULLOUS SPECTACULOPATHY IN A *BOIGA CYANEA*

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Summary

In summer 2014, a 10 year old male green cat snake (*Boiga cyanea*) with a weight of 664 g showed swelling of the spectacle of the left eye. The animal was anaesthetised with isoflurane, and proptosis explored and treated at our zoo. Under slit lamp exploration we observed an increase of spectacle space and the presence of a transparent fluid inside. A bullous spectaculopathy was suspected.

Introduction

A bullous spectaculopathy is a marked enlargement of the spectacle more commonly found in snakes and geckos than any other reptile. This distension of the spectacle, which may often be confused with glaucoma, is a condition normally caused by obstruction of the nasolacrimal ducts originated by congenital atresia, acquired physical obstruction, infectious aetiology, or ecdysis complications. In summer 2014, a 10 year old male green cat snake (*Boiga cyanea*) showed swelling of the spectacle of the left eye. The animal was anaesthetised and proptosis explored and treated at our zoo. Under slit lamp exploration an increase of spectacle space was found.

Material and methods

The green cat snake was induced in a chamber with isoflurane (Isovet[®] veterinary B. Brawn, Rubi, Barcelona, Spain) in a flow of 5 % for 5 min and the anaesthesia was maintained by endotracheal tube 2 mm in a 2 % flow during the surgery. Samples of the fluid were submitted for bacteriological culture and cytology and an infectious aetiology was diagnosed with the presence of *Stenotrophomonas maltophilia*. A paracentesis with a 20 G needle and fluorescein (colircusi fluoresceina Alcon^R) injection was performed in the dorsal spectacle under general anaesthesia. No fluorescein was found in the mouth and then a bullous spectaculopathy secondary to a nasolacrimal obstruction was diagnosed. The bullous spectaculopathy was resolved by a triangle excision performed in the ventral spectacle with a 2.75 angled knife and a Vannas scissor under surgical microscope.

The snake was treated with Enrofloxacin (Baytril 5 %[®] Bayer, Barcelona, Spain) 10 mg/kg once a day SC, Meloxicam (Metacam[®] 15 mg/ml suspension, Boehringer Ingelheim, Barcelona, Spain) 0.1 mg/kg per day, and tobramycin/dexamethasone suspension (Tobradex Alcon^R Alcon Cusí, Barcelona, Spain) twice a day during seven days.

Results and discussion

In this clinical case we describe the standard treatment to resolve the bullous spectaculopathy to excise a ventral triangle of spectacle, allowing continual drainage of tears until the next shed. This technique requires microsurgery and should be performed by a specialist veterinary ophthalmologist.



*Fig. 1: Left ocular proptosis or exophthalmos in a *Boiga cyanea*. (Photo: Acuña A)*



Fig. 2: Aspect of left eye after surgery and treatment. (Photo: Acuña A)

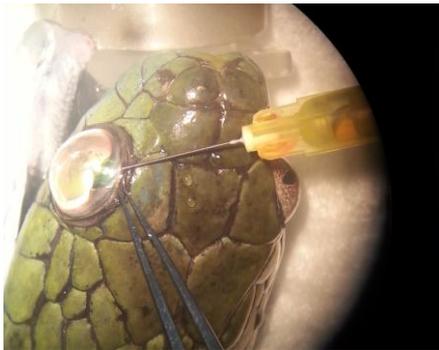


Fig. 3: Paracentesis with a 20G needle and fluorescein. (Photo: Martínez B)



Fig. 4: Diagnosis of a bullous spectaculopathy secondary to a nasaolacrimal obstruction. No fluorescein was found in the mouth. (Photo: Acuña A)



Fig. 5: Excision performed in the ventral spectacle with a 2.75 angled knife. (Photo: Acuña A)



Fig. 6: Triangle excision performed in the ventral spectacle with Vannas scissor. (Photo: Acuña A)

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USING TASMANIAN DEVIL BEHAVIOUR TO INDICATE ANIMAL BREEDING AND WELL-BEING IS A VITAL PART OF THE CAPTIVE MANAGEMENT PROGRAMME

ALBION I

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Tasmanian devils are the largest carnivorous marsupial remaining in the world. They are now endemic to Tasmania and in 1996 the first devil was recorded with what is now known as Devil Facial Tumour Disease. In 2004 Tasmanian devils were brought into captivity to manage this fatal, transmissible cancer outbreak.

For the first time, a co-ordinated captive breeding and management programme was undertaken in an attempt to save the Tasmanian devil. Initially, the captive management programme was mainly focused on breeding success and it soon became apparent there were many unknowns about both wild and captive behaviour of devils and their breeding behaviours. By recording all devil behaviours – from both indirect and direct observation, I was able to identify trends in breeding readiness and success. When devils simultaneously display five or more specific behaviours, the likelihood of successful breeding is very high whereas the success is much lower when the behaviours do not coincide or fewer are shown. The most successful breeders were wild-caught females which spent at least three days sharing the same den with a male, not eating and producing less than two scats per devil for up to six days. All successful females had access to a male throughout oestrus. In general, the devils bred in late summer or autumn, usually on their first oestrus as two year olds and showed typical birthing behaviour afterwards.

Oestrus cycles were recorded in 12 female devils between 2008 and 2014. Eight of these devils were wild-caught females bred with wild-caught males. The ninth was a wild orphan bred with a wild-caught male. All nine wild-caught females showed similar behaviours at oestrus and birthing and all but one successfully bred. Two that did not successfully birth came back into oestrus within two months and showed typical successful breeding behaviours. One also bred in successive years. The captive-bred females were more erratic in their breeding behaviour and were often unsuccessful breeders with only one successfully rearing young. Those that did not show any birthing behaviour did not come back into oestrus again until the following summer.

In summary, having observant, trained keepers to record all behaviours via direct and indirect devil observation has led to identification of important animal-based indicators. In this case they were used to identify breeding readiness and success in both wild-caught devils and captive-bred devils. Such techniques could also be applied to identifying individual personalities, behaviour and physical and mental well-being of captive animals.

LUNG WORMS OF WILD BOARS (*SUS SCROFA BARBARUS*) IN THE MIDDLE ATLAS REGION OF MOROCCO

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Although the helminth parasites of domestic hogs are well documented worldwide no information is available about the digestive and pulmonary helminth infections of wild boar in Morocco. The aim of this work was to investigate the parasitofauna of free-ranging wild boars living in the in the Middle Atlas region of Morocco. Prevalence of lung worms belonging to the genus *Metastrongylus* was surveyed on 30 wild boars (*Sus scrofa barbarus*) (19 females and 11 males) from four areas of El Hajeb province (Middle Atlas) hunted officially for wildlife damage control, from June 2014 to December 2014. The wild boars were necropsied immediately after death. The thoracic and abdominal viscera were isolated, removed, placed in plastic bags, labelled and taken to the laboratory for further research. Records were kept of their age (according to dental formula and physical appearance), gender and locality. The number of parasites was the highest in the respiratory tract especially in the lung lobes. They were removed and placed in vials containing fixative (10 % formalin). The most prevalent genus *Metastrongylus* spp. (Molin, 1861) was identified, 76.66 % of the boars were infected with this nematode genus. The prevalence and infection intensity was higher in younger animals (< 1 year old) than in older animals. There were no significant differences in prevalence between sexes.

LONG TERM ANTIBODY RESPONSES TO VACCINATION AGAINST AVIAN INFLUENZA IN BIRDS AT BARCELONA ZOO

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In 2006 and 2007, a number of European zoos implemented preventive avian influenza (AI) vaccination protocols during the H5N1 influenza outbreak which originated in SE Asia in 2003, to forestall putative AI outbreaks within their bird collections. Several studies of the short-term serological response to these vaccination programmes have been published, showing a heterogeneous response to the different vaccination protocols implemented as well as across bird taxa. However, no data are available on the long term antibody responses elicited by AI vaccination of zoo birds. Here we present preliminary results of a serological survey of AI antibodies in zoo birds seven years after vaccination.

In 2014, we performed an opportunistic sampling of birds which had been vaccinated against AI in 2006 and 2007 at Barcelona Zoo. Vaccination at that time followed the protocol set out by the Ministry of Agriculture, Food and Environment. It consisted of two IM doses of Poulvac Flufend[®] H5N9 (Fort Dodge Animal Health, Olot, Spain) in 2006, followed by an IM booster based on risk analysis (only in Phoenicopteriformes and Ciconiiformes) with Poulvac Flufend RG[®] H5N3 (Pfizer Animal Health) in 2007. Vaccinated birds belonging to three orders were sampled: Phoenicopteriformes (*Phoenicopiterus ruber*; $n = 21$), Ciconiiformes (*Platalaea leucorodia*, *Plegadis falcinellus*, *Bubulcus ibis* and *Ciconia ciconia*; $n = 21$), and Psittaciformes (*Ara ararauna*, *Ara rubrogenys*, *Ara militaris*, *Ara chloroptera*, *Amazona albifrons*, and *Aratinga guarouba*; $n = 24$). Unvaccinated birds ($n = 28$) from the same taxa, most of them born after 2007, were used as negative controls. Serological response was measured using ELISA against influenza A nucleoprotein and haemagglutination inhibition assay (HIA) for H5 (GD Animal Health, Deventer, The Netherlands).

All the control birds were negative by ELISA and HIA. Only two vaccinated birds were positive by ELISA but negative by HIA. Strikingly, all the vaccinated Phoenicopteriformes, and all but six vaccinated Ciconiiformes presented HIA titers ≥ 16 . Geometric mean titers (GMT) were 33 for Ciconiiformes and 124 for Phoenicopteriformes. By contrast, antibody levels among Psittaciformes were lower, and only 6 psittacines had HIA levels ≥ 16 (GMT = 9).

These results show persistence after seven years of the serological immune response to AI induced by vaccination in some zoo bird species. Further research is warranted to determine what are the antibody patterns in other taxa relevant in AI epidemiology, such as Anseriformes and Galliformes, and to confirm whether the weaker response in psittaciformes is due to the absence of the booster used in the other groups. Regardless, these data suggest that vaccination may induce long lasting immunity in some species. This finding has a strong importance in avian influenza risk assessment and contingency planning in zoos.

ENCEPHALITOOZON CUNICULI IN EUROPEAN BROWN HARES (LEPUS EUROPAEUS)

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Summary

Encephalitozoon cuniculi is an obligate intracellular parasite usually infecting domestic rabbits, although spontaneous infections have been documented in other mammalian species worldwide. Encephalitozoonosis is a chronic and latent disease leading to renal failure, encephalitis, disorders of brain and urinary tract and potentially to death. Samples of blood sera from 701 wild hares from the Czech Republic, the Slovak Republic and Austria were examined by indirect immunofluorescence (IFAT). Samples with a titer ≥ 40 were marked as positive. The total seroprevalence of *E. cuniculi* antibodies was 1.42 % with titres in the range 40 – 640. Antibodies to *E. cuniculi* were detected in 2.9 % (7/245), 0.8 % (2/245) and 0.47 % (1/211) hares from the Czech Republic, Austria and the Slovak Republic, respectively. This is the first detection of antibodies to *E. cuniculi* in hares, showing that hares are exposed to *E. cuniculi* infection.

Introduction

Encephalitozoon cuniculi was first identified in a group of laboratory rabbits in 1922 and currently is considered a zoonotic parasite (JORDAN, 2006). Encephalitozoonosis is a chronic and latent disease leading to renal failure, encephalitis, disorders of brain and urinary tract and potentially to death (DEPLAZES *et al.*, 2000). The most frequently infected animals are domestic rabbits (JEKLOVÁ *et al.*, 2010a,b), however spontaneous infections have been documented also in other domestic, wild and zoo animals. In 2007, *E. intestinalis* and *E. hellem* were diagnosed in the kidneys of a free-ranging European hare that was found dead in Belgium. The visible kidney lesions were similar to lesions caused by *E. cuniculi* in rabbits (DE BOSSCHERE *et al.*, 2007).

After infection, the specific antibodies to *E. cuniculi* could be detected by serological methods; these antibodies remain in animals throughout their life (DEPLAZES *et al.*, 2000). In Europe, antibodies to *E. cuniculi* were detected in various groups of domestic and wild animals but not yet in hares. The aim of this study was to examine the sera of hares from three European countries for such antibodies.

Material and methods

Blood serum from 701 wild European hares was serologically examined to determine specific *E. cuniculi* antibodies. These samples were collected in the Czech Republic ($n = 245$) from the Central Bohemia, South Moravian and Olomouc Region, from the Slovak Republic ($n = 211$) in the district of Nitra and from Austria ($n = 245$) from the federal lands of Lower Austria, Salzburg and Tyrol. All of the

hares were without clinical signs and their blood was withdrawn by hunters and veterinarians from their hearts. The sera were examined in the State Veterinary Institute, Prague, in the Czech Republic.

Serology

The sera were tested using the indirect immunofluorescence antibody test (IFAT) using the commercial set MegaScreen Fluencephalitozoon c. (Megacor Diagnostic, Hörbranz, Austria) in twofold dilution starting with the basic dilution of 1:40. The procedure in brief: 20 µl of serum was placed in each well of slides that were covered with *E. cuniculi* antigen. After incubation for 30 min at 37°C the slides were washed with PBS. Next, the wells were covered with anti-rabbit immunoglobulin fluorescein-isotiocyanate (FITC) conjugate (Santa Cruz Biotechnology's, Santa Cruz, USA) with the addition of Evans blue, and were incubated at 30 min for 37°C. They were then washed with PBS, air dried and mounted with 80 % glycerol. The slides were examined under an immunofluorescence microscope. In the case of positive samples, green fluorescence was visible; if there was a red coloration of antigen (tachyzoites of *E. cuniculi*), the sample was considered negative. The samples with titres ≥ 40 were considered positive. Positive and negative controls were included in the test.

Results and discussion

In our study, 701 samples of blood sera from hares were used for serological examination. Antibodies to *E. cuniculi* were detected in 10 (1.42 %) hares with titres in the range 40 – 640 (table 1). In the Czech Republic, Austria and the Slovak Republic antibodies were detected in 2.9 % (7/245), 0.8 % (2/245) and 0.5 % (1/211) hares, respectively.

Tab. 1: Seroprevalence and titres of *Encephalitozoon cuniculi* in European hares examined by indirect immunofluorescence (IFAT).

Country	Positive/ total samples (%)	IFAT (titre)				
		40	80	160	320	640
Czech Republic	7/245 (2.9 %)	1	5	1	–	–
Slovak Republic	1/211 (0.5 %)	–	–	1	–	–
Austria	2/245 (0.8 %)	–	–	–	1	1

The results of serological examinations may be variable due to many factors such as the number of animals included in the study, their gender and their age because the risk of infection increases with the age of animals (DIPINETO *et al.*, 2008). However, we could not evaluate these risk factors, because we do not have such data available. Since this is the first detection of *E. cuniculi* antibodies in hares, we can only compare our results with similar studies done on European rabbits (*Oryctolagus cuniculus*) belonging to the same order, Lagomorpha, or with other domestic or wild animals. In the Czech Republic, *E. cuniculi* seroprevalence in domestic animals is much higher than the prevalence obtained in hares from the present study. This can be explained by the fact that domestic animals are more likely to come into contact with rabbits (*Oryctolagus cuniculus*), which are the primary hosts of *E. cuniculi*. Rodents also play an important role in spreading this infection because they can

contaminate the water and food with *E. cuniculi* spores. Hares may be primarily infected by ingestion of food and water contaminated with these spores and, when they become prey for foxes or other carnivores, encephalitozoonosis can spread through other wild animals. In the Slovak Republic, the situation is similar to that in the Czech Republic, with a higher prevalence in domestic and other wild-life animals compared to the hares from the present study. In Austria, the prevalence of antibodies to *E. cuniculi* was only 6 % (16/268) in voles (*Microtus arvalis*) and 7 % (6/86) in European water voles (*Arvicola terrestris*) (FUEHRER *et al.*, 2010). Compared to these species, we detected much lower *E. cuniculi* prevalence in hares.

It is very important to obtain knowledge on the spread of *E. cuniculi* infection in meat animals, domestic animals and also in wildlife, because encephalitozoonosis is an important zoonosis. Antibodies to *E. cuniculi* were detected in humans from the Czech Republic and the Slovak Republic; in Austria data are not yet available.

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A ZOO ANIMAL WELFARE ASSESSMENT FORM: PRELIMINARY RESULTS OF FIELD IMPLEMENTATION

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Animal welfare assessment within zoos is a big challenge for public health veterinarians, who sometimes are called to face this topic, due to the number of species hosted in zoological gardens characterised by different physiological and ethological needs. Aim of the present study was to design a form intended to be used by the veterinary officers as a tool to survey the welfare status of animals in zoos and to test its application in the exhibitions. The form was constructed of the following three sections: an interview with general knowledge to a person in charge of animal management (12 questions), a behavioural observation of three different species (single animals or groups of animals) in the exhibitions (11 questions per species), and a general overview of the enclosures design and features (13 questions). Eleven vets, with different level of knowledge on wild animal welfare, from basic to deeper level, and six different zoological gardens situated in Piedmont region (Italy) were involved in this study. Between two and four forms were compiled per zoo, and a total of 20 documents were collected. Answers from the forms were analysed and the inter-observer reliability was calculated using a measurement of agreement (Cohen's kappa). The values of agreement ranged from $k = 0.4$ (CI95 % 0.28 - 0.53) to 0.9 (0.72 - 1). Results showed that even though the forms were compiled by veterinarians with different level of knowledge on wild animal welfare, the agreement was high. Therefore, this form could be an efficient tool for assessing welfare within zoos. It could then be used not only by veterinary officers during their duties, but also by zoo managers, which could implement it on a regular basis as an internal audit control.

**BEHAVIOURAL ANALYSIS OF BLUE AND YELLOW MACAW
(*ARA ARARAUNA*) UNDER DIFFERENT ZOO MANAGERMENTS:
PRELIMINARY DATA**

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Activity budgets of ten captive blue and yellow macaws (*Ara ararauna*) were analysed in order to assess their welfare. Birds were housed in four different zoological gardens, situated in Piedmont region, Italy. An ethogram of 33 behaviours was adapted from literature. An instantaneous focal animal sampling method was used to record behaviours with an interval of two minutes. Data were collected by five observers. The reliability of the observation method was assessed by Cohen's kappa, during two sessions carried out in two different days. The behaviours were categorised into signs of stress, of good welfare, and neutral: briefly, known stereotypies were considered signs of stress while playful and interactive behaviours as signs of good welfare. Each institution was visited five times and observations were taken when animals were on exhibition. The observers reached a within-observers reliability ranging from kappa = 0.6 (CI95 % 0.4 - 0.8) to kappa = 0.88, (CI95 % 0.76 - 1) and a between-observers reliability of kappa = 0.81 (CI95 % 0.76 - 0.85) for the first day of observation and kappa = 0.86 (CI95 % 0.81 - 0.9) for the second day.

Over 256 hours of data were collected and an amount of 7,700 observations were recorded. Analyses of the activity budget revealed that macaws spent 34.34 % of their time resting. Other frequently occurring behaviours were preening (15.35 %) and hanging beneath a perch (14.01 %). Major stereotypies observed were oral stereotypies (3.38 %) and head bob (0.64 %). The expression of behaviours indicating good welfare was different between animals, ranging from 54.57 % of total observations to 2.35 %. Statistical analysis will be carried out in order to identify environmental or social factors influencing behaviours in blue and yellow macaws suggesting good welfare.

CLINICAL PRESENTATION OF UROLITHIASIS IN ASIAN SMALL-CLAWED OTTERS (*AONYX CINEREA*)

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Urolithiasis is a common problem in both captive and free-ranging *mustelidae* and it has also been described in Asian small-clawed otters (*Aonyx cinerea*). Whereas detailed information is available regarding the epidemiology and the chemical composition of stones, our understanding regarding the clinical relevance and the clinical signs of urolithiasis in small-clawed otters are still limited.

Here we describe five cases of urolithiasis in Asian small-clawed otters from two different zoological institutions (table 1). All animals were adults, which correlates with findings in other studies. In contrast to other studies that did not find a gender predilection, our cases involved 80 % females. In the cases where only the kidneys were affected, urolithiasis was an incidental finding. However in the cases where both the kidneys and the bladder were affected, clinical disease occurred. Anorexia and depression were the primary clinical signs. In clinical cases urine analysis appears to be a useful diagnostic investigation. Furthermore in the two clinical cases computed tomography (CT) was helpful to evaluate the extent of urolithiasis and to diagnose additional pathologies. In animal no. 4 CT revealed not only multiple uroliths in the urinary bladder and renal pelves, but also signs of osteomyelitis in the upper maxillary bone and a severe abscess formation within the left retrobulbar space. In animal no. 5 CT combined with ultrasound revealed severe mineralisations of both renal pelves, mild pyelectasis, hydronephrosis on the left side and uroliths in the urinary bladder.

Tab. 1: Clinical presentation of five Asian small-clawed otters (*Aonyx cinerea*) with urolithiasis.

Animal no.	Age (years)	Gender	Blood and urine	Stones	Clinical appearance	Remarks
1	4	Female	Hyper-phosphataemia	Bilateral renal uroliths	Asymptomatic	Still alive
2	3	Female	Not performed	Unilateral renal uroliths	Asymptomatic	Severe bite wound on the leg
3	4	Female	Not performed	Bilateral renal uroliths	Asymptomatic	Metritis
4	6	Female	Moderate pyuria, severe haematuria	Bilateral renal and cystic uroliths	Anorexia, depression	Additionally severe infection of the eye and retrobulbar space, osteomyelitis of maxillary bone
5	4	Male	Proteinuria, glucosuria, anaemia, hyper-phosphataemia, azotaemia	Bilateral renal and cystic uroliths	Anorexia, depression, ulcerative stomatitis	Stone analysis: calcium oxalate

COMPARATIVE EXAMINATIONS OF REPRODUCTIVE ACTIVITY IN LYNX SPECIES (PRELIMINARY RESULTS)

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Like the domestic cat, many wild felid species follow a seasonal poly-oestrous cycle pattern, thus multiple litters may be dispersed throughout the year. In contrast, *Lynx* spp. appear to have a unique reproductive strategy amongst felids.

Three out of the four lynx species are strictly seasonal, mono-oestrous breeders, hence with only one pregnancy per year. The reason for this strict seasonality is that corpora lutea (CLs) do not regress completely after parturition. These persisting CLs stay functionally active outside breeding season and produce progesterone, and thus prevent any further cyclicity. However, one lynx species is different: the bobcat (*Lynx rufus*). The reproduction biology of bobcats can be ranked in between the domestic cat and the other three lynx species.

The bobcat is the only poly-oestrous *Lynx* spp. and thus if the first pregnancy in the season is not successful, a new cycle is induced with a new chance for a successful pregnancy during the same breeding season. Although the bobcat shows persisting CLs, like the other three lynx species, it is unknown yet, if they are functionally active outside breeding season.

The aim of our study is to evaluate the annual reproductive cycle of bobcats by high resolution ultrasonography and serum hormone analysis. In particular, we focus on the non-breeding season in winter and the two weeks of inter-oestrus period during breeding season from February until July. There is evidence that this phenomenon occurs in female bobcats up to three times in case of an unsuccessful pregnancy. Preliminary data obtained on ($n = 6$) female bobcats indicated an identical sonomorphology compared to Iberian (*L. pardinus*) and Eurasian lynx (*L. lynx*). Outside breeding season ($n = 4$, Dec), we detected persisting CLs on both ovaries. The serum progesterone (P4) was 3.3 ± 0.9 ng/ml, and the mean serum oestradiol (E2) was 0.7 ± 0.1 ng/ml. During the same time of the year the serum P4 in Eurasian lynx ($n = 7$, Nov-Dec) was almost twice as high (6.9 ± 4.5 ng/ml), P4 in serum of Iberian lynx was even five times as high (14.0 ± 6.6 ng/ml, $n = 11$, Dec) as in the bobcat. Serum E2 was less pronounced in Eurasian lynx (0.27 ± 0.1 ng/ml), whereas in the Iberian species E2 was highly elevated (1.4 ± 0.7 ng/ml). In summary, bobcats do have persistent CL outside breeding season, but low serum P4 levels are indicative for a limited steroidogenic capacity and thus could explain why in this *Lynx* species follicular activity may resume. More detailed studies on a larger number of bobcats throughout the year are planned.

Iberian lynx samples were kindly provided by Environmental Council of the Government of Andalusia and Iberian Lynx Conservation Breeding Programme.

SCHMALLEMBERG VIRUS IN ZOO RUMINANTS

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Schmallenberg virus (SBV), a new Orthobunyavirus of the family *Bunyaviridae*, emerged in August 2011 in north-western Europe and spread to most parts of Europe. Most infections are asymptomatic in adult ruminants but fever, milk drop and diarrhoea have been reported. SBV is responsible for congenital malformations in newborn calves, lambs and goat kids; it is suspected to cause abortion and early embryonal loss as well. The disease mainly affects domestic livestock but wild ruminants such as red deer (*Cervus elaphus*) and European bison (*Bison bonasus*) could also be susceptible.

To investigate susceptibility of ruminant species kept in zoos, serum samples from 45 animals of La Ménagerie du Jardin de Plantes (Paris, France) and SafariPark Beekse Bergen (Hilvarenbeek, Netherlands) were tested with a SBV-specific competitive ELISA technique. The first positive sample was taken from a blue wildebeest (*Connocchaetes taurinus taurinus*) in September 2011. Respectively around 80 % and 40 % of the tested ruminants of La Ménagerie and Beekse Bergen were seropositive. We report for the first time antibodies against SBV in bharal (*Pseudois nayaur*), springbok (*Antidorcas marsupialis*), sable antelope (*Hippotragus niger*) and western tur (*Capra caucasica*). A serum neutralisation test (SNT) confirmed the results in all the ELISA-positive samples. RT-qPCR testing revealed SBV genome in one serum sample from a blue wildebeest, suggesting viraemia can occur in this species. Although no clinical signs have been reported up to now, reproduction should be carefully monitored in zoo ruminants. Further investigations are required to determine if zoo ruminants could play a part in dissemination of the virus.

**PREVALENCE OF *TOXOPLASMA GONDII* ANTIBODIES IN
CAPTIVE ZOO ANIMALS AND SYMPATRIC PERI-URBAN PEST SPECIES
AT CORDOBA ZOO, SPAIN**

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Toxoplasmosis is a worldwide zoonotic disease caused by *Toxoplasma gondii*, which infects humans and most warm-blooded animals. In zoo animals, including endangered species, reproductive disorders and mortality have been frequently associated to *T. gondii* infections, representing re-emerging outbreaks of disease and economic losses. The aim of this study was to determine the seroprevalence of *T. gondii* antibodies in captive zoo animals and sympatric peri-urban pest species from the Cordoba Municipal Zoo Park (CMZP) (Southern Spain). Serum samples from 91 captive zoo animals, including 27 carnivores, 36 ungulates, 26 birds and 2 monkeys were collected during 2013. In addition, serum from 142 domestic pigeons (*Columba livia domestica*) and 41 rats (7 black rats (*Rattus rattus*) and 34 brown rats (*Rattus norvegicus*) were sampled in the CMZP. Sera were examined by modified agglutination test (MAT) for the presence of antibodies against *T. gondii* (> 1:25). The overall seroprevalence in captive animals was 28.6 % (26/91). Antibodies to *T. gondii* were found in carnivores (40.7 %; 11/27), ungulates (30.6 %; 11/36) and birds (15.4 %; 4/26), but not in monkeys (0/2). Seropositivity against *T. gondii* was significantly higher in rats 42.9 % (18/41) than in domestic pigeons 9.2 % (13/142). The results indicate that *T. gondii* infection is widespread in captive zoo animals, especially in carnivorous species, from the CMZP. The high seropositivity in wild felids (46.2 %; 6/13) could be of public health significance because of the potential of oocysts shedding. Also, *T. gondii* shedding is of animal health concern due to the high susceptible zoological species such as Marsupials and New World monkeys against the parasite. The seroprevalence obtained in rats suggests that these two species are good sentinels for the presence of oocyst shedding and may act as important reservoirs in the transmission of *T. gondii*. Therefore pest control programmes should be well defined and implanted in zoological collections.

FLOTAC: A HIGHLY SENSITIVE TECHNIQUE FOR PARASITOLOGICAL DIAGNOSIS IN ZOOLOGICAL COLLECTIONS

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FLOTAC methods are a series of multivalent copromicroscopic techniques based on centrifugal flotation of the faecal sample and subsequent translation of the top layer of the floating suspension. The initial development of FLOTAC was inspired from other flotation based techniques such as the McMaster and Wisconsin methods. However, a central feature of the FLOTAC techniques is that they provide counts of parasitic elements in large faecal aliquots. Additionally, the FLOTAC techniques are characterised by high sensitivity (detection limit of one egg per gram of faeces). Due to its high accuracy and sensitivity, FLOTAC is a validated method to detect parasitic infections in livestock animals.

The aim of this study was to investigate the prevalence of gastrointestinal parasites in mammals housed at three zoological gardens of central-southern Italy.

Between July and December 2013, a total of 54 pools of faecal samples were randomly collected from various primates, hoofed mammals, felines, mustelids, canids, rodents, marsupials and chiropterans.

All faecal samples were analysed using the FLOTAC technique.

In total, 42.5 % of the tested animals resulted positive to parasites as follows: *Toxascaris leonina* (20.0 %) was found in felines, *Trichuris* spp. (6.6 %) and Strongyles (6.6 %) in mustelids and *Trichuris* spp. (6.6 %), *Capillaria* (6.66 %) and pinworms (6.6 %) in carnivorous. Intestinal strongyles were prevalent in monogastric herbivores (50.0 %) and marsupials (66.6 %). Primates were infected by *Trichuris* spp. (20.0 %), *Strongyloides* (20.0 %) and ascarids (20.0 %). Ruminants resulted positive to gastrointestinal strongyles (31.2 %), *Nematodirus* (25.0 %), *Trichuris* (12.5 %), *Capillaria* (18.7 %), ascarids (6.2 %) and *Coccidia* (6.25 %). Rodents were found negative at the testing, they are not treated before.

Monitoring of the presence of parasites in zoo animals is fundamental to diagnose, control and prevent the spread of infectious and parasitic diseases among animals kept at zoo gardens to safeguard welfare and health of animals and humans.

HEALTH PROBLEMS IN HATCHLING LOGGERHEAD SEA TURTLES (*CARETTA CARETTA*) FROM A HEAD-STARTING PROGRAMME IN THE MEDITERRANEAN SEA

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Head-starting programmes have been widely used as a conservation tool to maximise survival in species under critical conservation condition. For instance, head-starting was considered decisive to recover the Kemp's ridley sea turtle (*Lepidochelys kempii*) population facing extinction in the 80's when it was catalogued as critically endangered by the International Union for Conservation of Nature (IUCN). Early in the summer of 2014 a female loggerhead turtle was discovered nesting in Alicante, Spanish Mediterranean basin. Due to episode novelty and the low possibilities of success in such a massive tourist beach, it was decided to intervene. Egg clutch of 129 was divided in two and relocated for natural incubation in a protected beach (89 eggs) and in artificial incubation (40 eggs) at the Oceanogràfic aquarium in Valencia. After the incubation period, a total of 102 hatchlings (79 % combined hatching success) were included into the head-starting programme. All the animals were kept under the same conditions following prior experiences and international guidelines.

Although part of the histopathological, bacterial and virological studies are still pending at the moment of writing this abstract, two epizootic outbreaks threatened the health of the neonates. An acute onset of anorexia, lethargy and bloating followed by death was controlled after aggressive therapy consisting of antibiotics, supportive care and management modifications (feeding strategy and housing facilities). *Post mortem* analyses showed severely enlarged bowels with large amounts of fluid faecal material inside. Histopathological findings revealed necrotising and haemorrhagic enteritis, with large amounts of bacterial colonies attached to the necrotic epithelium. *Shewanella putrefaciens*, *Escherichia coli*, *Vibrio* spp., *Aeromonas* spp and *Clostridium baratii* were isolated from different intestinal samples, which would be more indicative of a dysbacteriosis than a problem associated with a specific aetiology. *Herpesvirus* spp. was detected in some tissue samples but virus' role is still pending to be clarified. A second outbreak was featured by the massive infestation of the copepod *Balaenophilus manatorum*, which previously had been described as apparently harmless epibiont in sea turtles. A severe overload of copepods was found mainly on the ventral region of the animals, producing a combination of clinical signs (skin and scutes erosions or ulcers, physical distress and death in most severe cases). Main lesions consisted of ulcerative and necrotising dermatitis. Different therapeutic options were discussed for both, animal and facilities including fresh water, formalin, chlorine, and lufenuron treatments.

Occasionally, multiple granulomas were observed in lungs from selected individuals deceased during the whole period, some of them presenting intralesional fungal hyphae.

DIAGNOSIS AND SURGICAL TREATMENT OF AN AURAL CHOLESTEATOMA AND OTITIS MEDIA IN A MONGOLIAN GERBIL (*MERIONES UNGUICULATUS*)

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A 3 year old male pet Mongolian gerbil (*Meriones unguiculatus*) presented for scratching and alopecia of the right ear over two weeks. Clinical examination showed a mild head tilt. A computed tomography (CT) scan of the head showed an auricular mass partially filling the tympanic bulla. A partial ear canal ablation and lateral bulla osteotomy (PECALBO) was performed. The surgical technique was similar to the one described in rabbits but the surgical approach was smaller, the vertical canal was very short, so the dissection to access to the bulla was easier. However, the tympanic bulla was relatively big, and divided with a septum which made the whole bulla difficult to clean. Adapted material (lenses and microsurgery instruments) was needed to perform this surgery. We submitted the contents of the tympanic bulla for histopathology and microbiology. An aural cholesteatoma and *Staphylococcus pseudintermedius* infection sensitive to enrofloxacin were identified. Five days after surgery, the patient appeared comfortable with no head tilt or scratching of the right ear. Antibiotic therapy (Baytril (Bayer Corporation, Paris, FRANCE) 10 mg/kg BID) was continued for 21 days post-surgery. Three months later the gerbil is still alive without recurrence of clinical signs. Old gerbils have a high spontaneous incidence of cholesteatoma. Usually these are incidental findings at necropsy. This case is unique as we used a CT scan to identify the cholesteatoma and then successfully removed the mass. This case illustrates that diagnostic and therapeutic techniques commonly used in companion animals can be effectively applied to small rodents.

KETAMINE AND MEDETOMIDINE IN MARSICAN BROWN BEAR CAPTURED WITH LEG-HOLD SNARE AND THE USE OF BEHAVIOURAL PARAMETERS FOR CHOOSING A SAFE DOSAGE

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Nowadays it is clear that many factors combine to influence the response to anaesthesia. According to previous studies about some species such as pigs, dogs and rats, it is known that the emotional state of a subject conditions as much as the clinical situation. In a lot of domestic species (dogs, cats, horses, cattle, goats) the behavioural indicators have already been codified, in order to evaluate animal welfare.

The aim of this study is to analyse the behaviour of Marsican brown bear (*Ursus arctos marsicanus*) captured with leg holding snare, to define a stress level descriptive scale and to use it in order to have the future opportunity of consequently adapting anaesthetic choices.

On the basis of some remarks observed during more than 200 captures of adults and sub-adults subjects performed in the Abruzzo, Lazio and Molise National Park from 1990 to 2013, we have identified a range of behaviours and described a numerical scale.

The scale consists of four classes:

- score 1 calm, alert but not excited;
- score 2 slightly excited but little interested in external events;
- score 3 excited and strongly responsive;
- score 4 extremely excited and aggressive.

After the capture the bears have been darted with medetomidine (Zalopine[®], Orion Pharma Animal Health, Turku, Finland) at the dose of 0.04 - 0.35 mg/kg body weight and ketamine (Ketavet[®] 100, MSD Animal Health Srl, Milano, Italy) at the dose of 1.98 - 14.0 mg/kg body weight.

By applying the scale only on adults ($n = 43$), we have confirmed that there is a positive correlation between animal's stress level and the anaesthetic needs for a safe approach: with the increase of the score it was required a greater dose of medetomidine ($r = 0.37$, $P = 0.003$) and ketamine ($r = 0.43$, $P = 0.0008$), the two drugs administered in each capture. It has been shown that stress may have some effects on the distribution and metabolism of sedative and anaesthetic drugs.

In the same group of animals, Spearman's rank correlation analysis has demonstrated that the stress score also has a significant positive correlation both with heart rate ($r = 0.33$, $P = 0.04$) and body temperature ($r = 0.38$, $P = 0.015$), some of the physiological parameters measured 15 ± 5 min after darting.

The need of greater anaesthetic doses in stressed animals is reported in literature for many species. In particular, medetomidine is known to act on catecholamine release and re-uptake: high concentrations of epinephrine and norepinephrine in stressed animals may influence its anaesthetic efficacy.

Certainly this type of evaluation can be useful in understanding the behaviour of leg-hold captured bears, protecting operators' safety and reducing the risk of perianaesthetic complications, considering the scarceness of Marsican brown bear population.

ECHOCARDIOGRAPHIC PARAMETERS IN ADULT WILD BORN CAPTIVE CHIMPANZEES (*PAN TROGLODYTES*)

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Heart disease is the leading cause of mortality among captive chimpanzees. Echocardiography is a non-invasive tool used to assess cardiac health. At present standard echocardiographic reference intervals for chimpanzees do not exist. A total of 113 wild born apparently healthy adult (> 10 years in males and > 15 years in females) chimpanzees underwent health assessments at two African sanctuaries. The animals were anaesthetised using four different anaesthetic protocols: Medetomidine (0.03 mg/kg, Dormitor[®], Pfizer Animal Health, Kent CT139NJ, UK) – Ketamine (3 mg/kg, Kyron Laboratories, Benrose, Johannesburg 2094, SA) ($n = 47$), Tiletamine-Zolazepam (2 mg/kg) - Medetomidine (0.03 mg/kg) ($n = 22$), Tiletamine-Zolazepam (10 mg/kg, Telazol[®] Wyeth, Madison, NJ 07940, USA) - Ketamine (3 mg/kg) ($n = 18$) and Tiletamine-Zolazepam (10 mg/kg) ($n = 15$). As part of the health assessment a transthoracic echocardiogram was completed. Measures of cardiac structure (cavity dimensions, wall thicknesses and mass), systolic function (ejection fraction) and diastolic function (early [E] and late [A] trans-mitral blood flow velocities) were assessed. Good quality images were obtained from 102 (64 females and 38 males) animals. Reference intervals were calculated as the 5th to 95th percentiles. Differences between male and female chimpanzees were assessed using independent T-tests with alpha set at 0.05. There was no significant difference in age (age; [mean \pm SD] 22 \pm 5 yrs vs. 19 \pm 6 yrs); however, males had greater body mass (54.5 \pm 8.1 vs. 42.3 \pm 7.2 kg, $P < 0.05$). As expected all structural parameters were smaller in females ($P < 0.05$). Reference intervals for cardiac structure and function are provided in table 1. Animals presenting with cardiac structure or function outside of these intervals should be considered for longitudinal monitoring.

Tab. 1: Normal echocardiographic reference intervals for adult male and female chimpanzees.

LV Structure and function	Mean \pm SD		5 th - 95 th Percentile Range	
	Males ($n = 38$)	Females ($n = 64$)	Males	Females
Septal diameter (d) (cm)	0.82 \pm 0.11	0.69 \pm 0.11*	0.62 - 1.00	0.50 - 0.90
Left ventricle (d) (cm)	4.86 \pm 0.44	4.18 \pm 0.33*	4.10 - 5.71	3.53 - 4.70
Left ventricle (s) (cm)	3.74 \pm 0.47	3.03 \pm 0.43*	2.66 - 4.40	2.13 - 3.62
Posterior wall (d) (cm)	0.87 \pm 0.14	0.68 \pm 0.12*	0.60 - 1.11	0.50 - 0.90
Relative Wall Thickness	0.36 \pm 0.06	0.33 \pm 0.06*	0.24 - 0.48	0.23 - 0.43
Ejection Fraction (%)	54 \pm 6	57 \pm 6*	43 - 66	46 - 67
E/A Ratio	2.14 \pm 0.77	2.12 \pm 0.81	0.81 - 3.48	1.10 - 3.87

*indicates significantly different from males ($P < 0.05$). LV = left ventricular, d = diastole, s = systole, mid = mid wall

SALMONELLA ABORTUSOVIS INFECTION IN CAPTIVE WILD CAPRINES: SURVEY AND HEALTH MANAGEMENT

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Summary

Salmonella enterica subsp. *enterica* serovar abortusovis is a major cause of reproductive failure in domestic small ruminants. Following several reproductive problems in a population of Himalayan blue sheep (*Pseudois nayaur*) at the Ménagerie du Jardin des Plantes in Paris, France, serological analyses revealed the presence of *Salmonella* abortusovis. The study focused initially on all Caprini presented at the Ménagerie before including animals from partner institutions in France and the Netherlands. An initial serological survey using agglutination by micro-titration on 91 caprine sera was carried out. Further analyses on a larger population are ongoing. The study of neonatal morbidity and mortality in the caprine collection at la Ménagerie du Jardin des Plantes over the last ten years shows that 21 % of all viable caprine neonates died in the first week of their life. Thirteen out of 91 sera tested positive by micro-agglutination: the infection by *Salmonella* abortusovis is present in the study collection. We advocate that serological tests of new arrivals in collections, vaccination in selected cases and strategic health monitoring procedures are to be discussed as preventive sanitary measures when dealing with captive wild caprine collections.

Introduction

Salmonella enterica subsp. *enterica* serovar abortusovis (TINDALL *et al.*, 2005; GRIMONT and WEILL 2007; AGBAJE *et al.*, 2011) is a major cause of reproductive failure in domestic small ruminants. Colonisation of the foeto-placental unit in sheep and goats causes abortion as well as neonatal morbidity and mortality. Massive vaginal excretion of *Salmonella* abortusovis in the postpartum period is considered to be the main source of contamination of the environment (PARDON *et al.*, 1988; CAGIOLA *et al.*, 2007; VALDEZATE *et al.*, 2007).

Following several reproductive problems in a population of Himalayan blue sheep (*Pseudois nayaur*) at the Ménagerie du Jardin des Plantes in Paris, France, serological analyses revealed the presence of *Salmonella* abortusovis.

The aim of this study is to determine whether *Salmonella* abortusovis is involved in reproductive failure in captive wild caprine populations and determine a management plan for positive individuals in zoological collections.

Materials and methods

The study focused initially on all Caprini presented at the Ménagerie before including animals from partner institutions in France and the Netherlands. An initial serological survey using agglutination by micro-titration on 91 caprine sera was carried out (25 simple sera taken from on-site animals in 2014

and 66 simple sera from different serum banks representing animals from the study collection over the last ten years). Further analyses on a larger population are ongoing.

Moreover, a survey was distributed among the members of the French Association of Zoo Veterinarians in order to assess the importance of ovine salmonellosis in French zoological institutions.

French departmental sanitary farmers associations are also being contacted in order to add their expertise in domestic flocks as well as epidemiological data to the current study.

Results

The study of neonatal morbidity and mortality in the caprine collection at la Ménagerie du Jardin des Plantes over the last ten years shows that out of 138 viable caprine neonates, 30 died in the first week of their life (21 %). Compared to the global mortality of caprines in the same period, almost 40 % of the deaths occur before ten days of age (figure 1).

Initial test results show that *Salmonella abortusovis* infection is indeed present in captive wild caprines. Thirteen out of 91 sera tested positive by micro-agglutination.

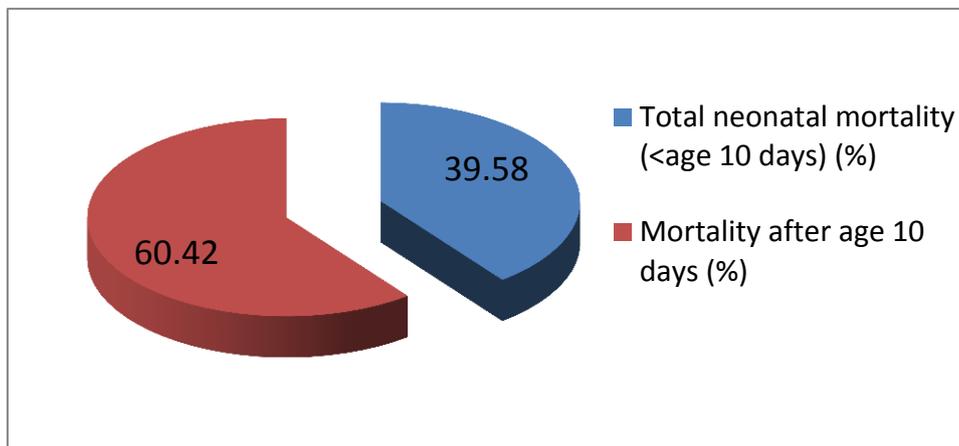


Fig. 1: Neonatal mortality compared to global mortality in seven caprine species showing incidence of neonatal morbidity and mortality between 2003 and 2013 at the Ménagerie du Jardin des Plantes in Paris, France.

Discussion

In Europe, several health care studies on domestic flocks show a high prevalence of *Salmonella abortusovis* infection.

Pioz *et al.* (2006, 2008a, b) demonstrated the implication of *Salmonella abortusovis* in reproductive failure in wild chamois (*Rupicapra rupicapra*) and Alpine ibex (*Capra ibex ibex*) and its transmission between domestic small ruminants and wild sheep and goats.

The preliminary results of our study indicate that infection by *Salmonella enterica* subsp. *enterica* serovar *abortusovis* is present in the study collection and is thus an issue for reproductive management of the species concerned. Though we cannot yet statistically prove a significant association between the important incidence of neonatal morbidity and mortality and the infection by *Salmonella abortusovis*, we advocate that serological tests of new arrivals in collections, vaccination in selected cases and strategic health monitoring procedures are to be discussed as preventive sanitary measures when dealing with captive wild caprine collections. Further tests of does and ewes at the

time of birthing and analysis of placental tissue are warranted to confirm the preliminary findings and prevent further reproductive losses.

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USE OF IMPLANTABLE CARDIAC DEVICES FOR MONITORING HEART HEALTH IN GREAT APES

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Three adult male western gorillas (*Gorilla gorilla gorilla*) and five adult chimpanzees (*Pan troglodytes*) (one male and four females) were implanted with Medtronic Reveal XT 9529 devices. Implants were placed in one of two locations; dorsally just medial to the scapula in the gorillas and ventrally either subcutaneously or under the edge of the pectoralis muscle in the chimpanzees. Three of the devices placed subcutaneously in the ventral location were lost due to removal by conspecifics or development of implant site dehiscence. Devices were interrogated to collect data every 2 to 4 weeks. Day and night time heart rates, activity level and heart rate variability were recorded and all arrhythmic episodes were evaluated for authenticity and logged in a spreadsheet. Heart rate variability values and activity levels were similar to those seen in healthy humans, and may be useful for detection of heart failure. Daytime resting heart rates for gorillas ranged between 65 and 81 beats per minute. One of the gorillas has a low resting heart rate and is prone to recorded episodes of bradycardia, especially while sleeping. Chimpanzees were especially prone to tachyarrhythmia detection when standard human programming was used. The two female chimpanzees had periods of tachyarrhythmia with a mean heart rate of 154 bpm and 197 bpm, and average maximum heart rates of 172 bpm and 199 bpm. In 2014 Medtronic introduced a smaller Linq implantable cardiac device to the market. A single male chimpanzee has received this new implant, and early data shows better ECG sensing and fewer false detections.

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THE SABAH RHINO (*DICERORHINUS SUMATRENSIS HARRISSONI*) BREEDING PROGRAMME: CURRENT SITUATION AND FIRST CONCLUSIONS

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The Sumatran rhinoceros (*Dicerorhinus sumatrensis*) is on the verge of extinction. Once found throughout Southeast Asia, it stands now with less than 100 individuals scattered mainly in three national parks in Sumatra. In Sabah (Malaysian Borneo), as well as in Peninsular Malaysia, the Sumatran rhinoceros is now considered to be “functionally extinct”. Between 1984 and 2014, 45 Sumatran rhinoceroses were captured from the wild, resulting in only two successful breeding pairs. In early 2015 only nine individuals survive in captivity, three (1.2) in the Borneo Rhino Sanctuary, in Sabah; five (2.3) in the Sumatran Rhino Sanctuary, in Sumatra; and only one (1.0) left in Cincinnati Zoo, USA. Since 2009 the Leibniz Institute for Zoo and Wildlife Research (IZW) has been collaborating with the Borneo Rhino Alliance (BORA), mainly through the use of advanced imaging and assisted reproduction technologies on the 1.3 wild-caught Bornean rhinoceroses (*Dicerorhinus sumatrensis harrissoni*) held at the Borneo Rhino Sanctuary. Throughout 14 visits to Sabah, the IZW team and associated experts had the chance to perform 42 reproductive assessments, six semen collections by electroejaculation, three endometrial cyst removal procedures, one artificial insemination (AI), three oocyte collections (“ovum pick-up”, OPU) and one intracytoplasmic sperm injection (ICSI). In what concerns the male, significant improvements in semen quality were observed throughout time: despite being considered reproductively inactive in 2009 and 2011, in 2014 a maximum concentration of 5×10^6 sperm cells /ml was obtained, with 65 % motility. When captured from the wild, two 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. Four different techniques were used to remove endometrial cysts: uterine lavage with cell medium M199 (Sigma-Aldrich Chemie GmbH, Munich, Germany) and povidone-iodine solution (Braunol[®], B. Braun Melsungen AG, Germany), endoscopic assisted laser photoablation and ultrasound guided aspiration. These procedures proved to be of very limited success. Consequently, AI was attempted only once and no fertilisation occurred due to poor condition of the uterus and irregular cycling. As natural conception was excluded, *in vitro* fertilisation was attempted for the first time in Sumatran rhinoceros. OPU was performed on three occasions and successful on two, with the total collection of five oocytes. ICSI was performed with two of the oocytes, but cleavage was not achieved. Furthermore, to preserve the genome of these that may well be the last Bornean rhinoceroses, cell cultures were established from skin and mucosal samples and cryopreserved until seven passages, for future development of induced pluripotent stem cells.

EEHV IMPACT ON THE EUROPEAN POPULATION OF ASIAN ELEPHANT (*ELEPHAS MAXIMUS*): A PREVALENCE STUDY

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Since the first reported case in 1988, elephant endotheliotropic herpesvirus (EEHV) has claimed the life of 23 Asian elephant (*Elephas maximus*) calves in European captive institutions, and more than 50 under human care worldwide. This highly fatal disease is responsible for the death of 80 % of all positive EEHV cases. It presents itself as an acute haemorrhagic syndrome due to vast endothelial destruction, causing sudden death. The majority of fatal cases occur in young Asian elephants, especially ranging from one to three years of age.

To try to understand and quantify the impact of EEHV disease in the Asian elephant EEP (European Endangered Species Program) population, a case-controlled study was conducted. This lifetime prevalence analysis was based on retrospective data reported on the European Studbook, updated until the 1st of January 2015, with 72 institutions holding 298 (84 males, 214 females) Asian elephants. A similar epidemiological risk assessment had been previously accomplished for the North American captive Asian elephant population, and the lack of analogous results for the European population made this survey imperative.

Here we present the preliminary results of the prevalence studies at hand, which are included in a PhD project that aims at finding and understanding the most important factors that predict the likelihood of an elephant to die from the fulminant and devastating EEHV disease.

PATHOLOGICAL FINDINGS IN WILD BIRDS ADMITTED TO A REHABILITATION CENTRE IN THE VENETO REGION (NORTHERN ITALY): PRELIMINARY RESULTS

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Necropsy is an important component of avian medicine which requires a systematic approach for the examination of organs and the collection of samples. Very little is known about the variety of pathological changes which can be found in wild birds, and *post mortem* examination may help to understand the cause of death in these birds. Necropsies were performed on 52 wild birds admitted to a rehabilitation centre located in the province of Rovigo (Northern Italy) from February 2014 to January 2015. Dead birds belonged to the following species: *Falco tinnunculus* ($n = 15$), *Athene noctua* ($n = 8$), *Asio otus* ($n = 4$), *Ardea cinerea* ($n = 3$), *Picus viridis* ($n = 3$), *Ixobrychus minutus* ($n = 2$), *Columba palumbus* ($n = 2$), *Bubulus ibis* ($n = 1$), *Sturnus vulgaris* ($n = 1$), *Ardea purpurea* ($n = 1$), *Ardeola ralloides* ($n = 1$), *Merops apiaster* ($n = 1$), *Garrulus glandarius* ($n = 1$), *Pica pica* ($n = 1$), *Falco subbuteo* ($n = 1$), *Larus michahellis* ($n = 1$), *Otus scops* ($n = 1$), *Accipiter nisus* ($n = 1$), *Buteo buteo* ($n = 1$), *Tyto alba* ($n = 1$), *Rallus aquaticus* ($n = 1$). The most frequent pathological finding was severe muscle (mainly *pectoralis major*) atrophy probably due to starvation, followed by trauma (mainly wing and foot fractures), necrosis of the wings and the feet, and visceral gout consequent to renal failure and probably caused by dehydration. Rarely, necrotic foci were detected in liver, spleen and kidneys. Round (e.g. *Syngamus trachea*) and tape worms (identification is ongoing) were frequently found in respiratory (i.e. nares, trachea, bronchi and lungs) and digestive (i.e. oropharynx, stomach and intestine) tracts. In some cases, neither pathological changes nor lesions consistent with a specific disease were detected at necropsy. To note, wing necrosis affected 60 % of *Falco tinnunculus*, while 40 % showed feet necrosis. Both lesions were concurrently present in 33 % of affected birds. Furthermore, out of three necropsied *Picus viridis*, two showed a severe tape worm infestation. *Post mortem* examination is helpful in understanding the pathology of wild birds, but not always it can rule out the cause of death. Therefore, laboratory examinations should be always employed when investigating on the causes of mortality of wild birds.

SCREENING FOR INFLUENZA A VIRUS AND PARAMYXOVIRUS TYPE 1 IN WILD BIRDS ADMITTED TO A REHABILITATION CENTRE IN THE VENETO REGION (NORTHERN ITALY): PRELIMINARY RESULTS

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Wild birds are known to be the natural reservoir of avian influenza viruses and Paramyxoviruses. From February 2014 to January 2015, wild birds admitted to a rehabilitation centre in northern Italy were screened for the presence of these viruses. In total, 134 wild birds belonging to the species *Falco tinnunculus* (28 subjects), *Athene noctua* (27), *Apus apus* (12), *Asio otus* (10), *Buteo buteo* (7), *Picus viridis* (4), *Ardea cinerea* (3), *Larus michahellis* (3), *Turdus merulas* (3), *Garrulus glandarius* (3), *Columba palumbus* (3), *Columba livia* (3), *Otus scops* (3), *Tyto alba* (3), *Ixobrychus minutus* (3), *Streptopelia decaocto* (3), *Corvus cornix* (2), *Sturnus vulgaris* (2), *Circus aeruginosus* (2), *Ichthyaetus melanocephalus* (1), *Falco subbuteo* (1), *Ardeola ralloides* (1), *Bubulcus ibis* (1), *Merops apiaster* (1), *Pernis apivorus* (1), *Podiceps cristatus* (1), *Perdix perdix* (1), *Gallinula chloropus* (1), *Regulus regulus* (1) were sampled. Oropharyngeal and cloacal swabs, as well as serum samples were collected from each live bird, whereas viscera (i.e. brain, heart, kidney, lung, and intestine) were sampled from 47 birds died during the hospitalisation in the rehabilitation centre. All samples were submitted to the National Reference Laboratory for Avian Influenza and Newcastle Disease to be processed by molecular, virological and serological methods. All sera were tested using a competitive ELISA for influenza A and the Haemagglutination Inhibition (HI) test for Avian Paramyxovirus type 1 (APMV-1). Both the organs and the oropharyngeal and cloacal swabs were analysed by real-time RT-PCR to detect avian influenza viruses type A, while RT-PCR was used to detect APMV-1 viruses. Positive samples were further examined by virus isolation in embryonated SPF chicken eggs and cell cultures. No positivity for avian influenza viruses was detected, whereas three birds, one common kestrel (*Falco tinnunculus*), one common buzzard (*Buteo buteo*) and one common swift (*Apus apus*), resulted positive for APMV-1 viruses. Viral typing showed that the strains identified in common kestrel and in common swift originated from Newcastle Disease live vaccines. The virus identified in common buzzard was an APMV-1 strain commonly circulating in wild pigeons and doves (PPMV-1). Feeding common kestrel with poultry contaminated by live attenuated Newcastle Disease vaccines may have been the cause of infection during the hospitalisation in the Rehabilitation Centre, whereas the common swift could have been infected in the surroundings of a poultry farm. Since pigeons are natural preys of common buzzards, this could explain how the bird found positive for PPMV-1 could have been infected with the pigeon PMV-1 variant. These findings suggest that rescued wild birds may play a role in the diffusion of Avian Paramyxovirus virus type 1.

MANAGEMENT OF UNEXPOSED FRACTURES IN DEER BY THE USE OF A POLYURETHANE RESIN IMPREGNATED FIBERGLASS TAPE: A CASE SERIES

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In our wildlife rescue service facilities, cervids are the most common injured patients rescued on road accident. Patients such as roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*) are difficult to manage as adults. Surgery is commonly performed in injured calves, but post-operative management of wild animals may sometimes be difficult.

This work describes unexposed fractures management by the use of a polyurethane resin impregnated fiberglass tape (3M Vetcast Plus, 3M Animal Care, St. Paul, MN, USA) in five deer.

Four roe deer and one red deer were diagnosed for carpus, metacarpus, metatarsus and finger bone (two animals) unexposed compound fracture by radiographic assessment.

For applying the synthetic casting, all animals were deeply sedated by hand injection or using a jab stick: medetomidine (Sedator[®], Dechra Veterinary Products S.r.l, Torino, Italy) and ketamine (Ketavet[®] 100, MSD Animal Health Srl, Milano, Italy) combination was used IM at different doses, based on the species requirement (roe deer: medetomidine 0.05 mg/kg, ketamine 1.5 mg/kg; red deer: medetomidine 0,01 mg/kg, ketamine 3 mg/kg). Atipamezole (0.1 mg/kg; Atipam[®], Dechra Veterinary Products S.r.l, Torino, Italy) was administered IM at the end of the procedure to reverse the anaesthetic effect. Carprofen (Rimadyl[®], Large Animal Solution, Pfizer Ltd., Sandwich, UK) was given IM at a dose of 1 mg/ kg every other day for one week.

The bandage was held for 45 days; during this period, all animals were kept in a small outdoor enclosure where disturbance was kept to a minimum to reduce stress and the risk of further trauma.

The cast was removed under sedation when the formation of a stable callous was radiographically confirmed. All fractures healed well with good alignment.

After cast removal, all animals were moved to a larger area to allow more exercise to regain muscle strength. A further radiographical and clinical assessment was done before final release.

Conservative management of fractures in deer, consisting in rest and minimum disturbance, is considered by some authors sufficient for many fractures to heal, although injured deer remain active and this may negatively affect fracture repair.

Nevertheless, these two approaches may lead to non-union, mal-union or other problems. Surgical fixation is another approach, but it may require prolonged aftercare and lengthy captivity; moreover, surgical implants need subsequent surgical removal, which prolongs the recovery period. The use of a rigid bandage with specific characteristics such as lightweight, waterproof, endurance and strength, allows the animal to live in enclosures with other animals in a natural-like condition, reducing captivity stress, respecting animal welfare and enhancing fracture healing.

CARDIAC MONITORING OF JUVENILE MHORR-GAZELLES (*NANGER DAMA MHORR*) AT TIERPARK HELLABRUNN, GERMANY

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Mhorr-gazelles, *Nanger dama mhorrr*, originate from Northern Africa and are listed as “critically endangered” by the International Union for Conservation of Nature (IUCN). The wild population consists of less than 2,500 animals. Therefore a coordinated offspring management was initiated by the European Endangered Species Program (EEP) at the beginning of the 1970s.

Tierpark Hellabrunn has been a member of the EEP since 1981 and is currently housing 8,13 animals. Every year about six to ten offspring are born.

In 2011 clinical problems in combination with weak animals were noted for the first time. Six out of 10 juveniles born in 2011 had a cardiac murmur at auscultation *post partum*, four were born very weak and two of these died within the first 48 h after birth. In these two dead animals, necropsy revealed anatomical disorders including a patent foramen ovale (PFO) and patent ductus arteriosus (PDA) or a defect in septum interatriale. Retrospectively we analysed the necropsy reports of five young animals that have died between 2003 and 2012 and in three out of these five animals, a PFO and a PDA was reported. In one animal an omphalophebitis was also reported in necropsy, in other animals only the heart findings were described.

A genetically background was assumed and further questions were raised for the ongoing diagnostics: How can these cardiac findings be classified and validated? Is there a problem within the herd of mhorr-gazelles at Tierpark Hellabrunn, or does it extend onto the whole mhorr-gazelle population of the EEP?

For clarification of these questions, animal data and pathology results of the mhorr-gazelles at Tierpark Hellabrunn were analysed as well as the genetically background of the EEP.

Heart ultrasound screenings were conducted on all remaining juvenile mhorr-gazelles at Tierpark Hellabrunn to reveal more abnormalities. Cardiac monitoring was also conducted on the offspring generation of 2012. In 2011/2012 a total of 15 animals were checked by cardiac ultrasound. In over 60 % of these animals a clinical cardiac murmur was audible at the first health check after birth due to a physiological PDA still existing in the first days *post partum*. This cardiac murmur as well as the PDA disappeared in all checked animals until the 4th to 6th week *post partum*. At this time of age, none of the surviving animals showed any pathological finding in the ultrasound check. A possible relation between the FOP's mentioned in the necropsy reports of deceased animals and certain females in the population could be identified. The correlation needs to be proved over the next generations.

In the future all young mhorr-gazelles which will be born in Hellabrunn will be check routinely by heart ultrasound at the age of four to six weeks, to eliminate possible genetic reasons within this small population.

STANDING SEDATION IN SUMATRAN ELEPHANTS (*ELEPHAS MAXIMUS SUMATRANUS*) USING DETOMIDINE AND BUTORPHANOL

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Standing sedation using a modified protocol described for African elephants was conducted for comprehensive health and reproductive assessment and for minor clinical procedures in captive Sumatran elephants (*Elephas maximus sumatranus*). Thirty-three (4, 29) animals were investigated within three days. The elephants (body weight ranged from 650 to 2.800 kg) were managed in free contact hence save access to ear veins was possible in all animals, except in one aggressive bull. However, none of the animals was conditioned to receive trans-rectal ultrasonography. Therefore standing sedation was imperative. An initial hand-injection of detomidine (Domosedan[®], Janssen-Cilag GmbH, Neuss, Germany) hydrochloride and butorphanol tartrate (Alvegesic[®], CP-Pharma Handelsgesellschaft mbH, Burgdorf, Germany) was administered intravenously (i.v.) with a dosage of 10 - 40 mg/animal (10 – 25 µg/kg, mean 15 µg/kg) and 20 - 50 mg/animal (14 - 60 µg/kg, mean 21 µg/kg), respectively. The initial injection resulted in adequate sedation for initiation and completion of all procedures. No supplemental doses were required. The aggressive bull, which was not accessible for i.v. injection, received initially 60 mg (34 µg/kg) detomidine and 60 mg (34 µg/kg) butorphanol intramuscularly (i.m.). A supplemental injection i.v. of 10 mg (5.7 µg/kg) detomidine and 20 mg (11.4 µg/kg) butorphanol after 15 min was required to induce full sedation. Maximal effect occurred at 5 and 20 min after i.v. or i.m./i.v. application, respectively. All animals stopped moving and were standing steady with head down, trunk and tail fully immobilised. They were able to keep balance without signs of ataxia. No cardiac or respiratory depression was distinguishable. At 30 – 40 min after initial administration of the sedative, atipamizole (Antisedan[®], Janssen-Cilag GmbH; 10 – 30 mg per animal, mean 8 µg/kg, SD 4 µg/kg) and naltrexone (Naltrexon 50 mg/ml, S. Quandt, Johannesburg, RSA; 12.5 - 50 mg/animal, mean 18.3 µg/kg, SD 8.1 µg/kg) were applied i.v. (two third) and i.m. (one third). This resulted in rapid and complete recovery within 2 to 15 min. Animals diagnosed with kidney failures sonographically showed slower recovery (29 to 30 min). In contrast to former results were the authors used xylazine and yohimbine i.v. for short-term standing sedation in Asian elephants in Thailand no adverse side effects on gastrointestinal tract (e.g. anorexia, abdominal distention or bloat) could be observed this time. The combination of 10 µg/kg detomidine and 20 µg/kg butorphanol i.v. is highly recommended for standing sedation in Sumatran elephants. From the author's experience from other Asian and African elephants using detomidine and butorphanol in higher dosages as premedication with subsequent full immobilisation/anaesthesia in lateral recumbence (not included in this study) ataxia was observed with a dosage > 25 µg/kg detomidine in combination with butorphanol > 40 µg/kg body weight i.v.. This relatively high therapeutic index approves a safe and effective use of this protocol when body mass is not known and has to be approximated. The total dosage and induction time were reduced when detomidine and butorphanol was administered i.v., compared to i.m. administration.

THE IMPORTANCE OF MEDICAL TRAINING IN CAPTIVE ANIMALS: THE CASE OF FILIPPO, THE RED AND BLUE MACAW (*ARA CHLOROPTERA*)

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Animals with chronic diseases should be content every time they need medications, increasing the stress that could worsen an already serious situation. The training of an animal to perform preventive health behaviours helps to maintain the physical and psychological welfare of our animals. In fact, the body responds to various situations not only with behavioural changes but also with physiological and immune mechanisms, which may have an impact on the general health status. It is therefore important to introduce simple behaviours in the routine of the animal as an extra advantage because it shortens the operating time of the veterinarian, further reducing the level of stress. An example is Filippo, a red and blue macaw (*Ara chloroptera*) arrived at Zoomarine in 2006. The animal showed difficulty in breathing with asthmatic symptoms. During celioscopy, the presence of several black spots in the lungs, and over other serosal surfaces, were observed. This is commonly diagnosed as anthracosis. A chronic pneumonia was confirmed at biopsy and subsequent histopathology. At the occurrence of such symptoms, veterinarians and trainers intervened administering the *spray* VENTOLIN[®], (GlaxoSmithKline S.p.A., Verona, Italy) through a training designed to voluntarily assume the medicine. This behaviour was set out in various steps without ever forcing the animal. The issue of animal welfare can often be achieved only with a multidisciplinary approach that should also include medical behaviours.

DIFFERENT TECHNIQUES OF BLOOD SAMPLING IN SEALS (*PHOCA VITULINA*, *HALICHOERUS GRYPUS*) BY VOLUNTARY BEHAVIOUR: THE EXPERIENCE OF ZOOMARINE ITALIA

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Blood sampling is one of the most important clinical examinations to check the health of the animals. By using operant conditioning based on positive reinforcement, animals can be trained to voluntarily participate in welfare behaviours for minimising the stressful circumstances. All pinnipeds at Zoomarine Italia are trained for blood sampling. In our two species of seals (*Phoca vitulina* and *Halichoerus grypus*) we used four different techniques to take blood in and outside the water:

1. Interdigital: from interdigital veins located between fingers on dorsal and ventral face of hind limbs (using a 23G Butterfly System). On the base of our experience we preferred to stop using this technique. Being the interdigital vessels very small, this method is less effective respect time range, amount of sample and comfort of the animal, compared with the following three techniques.
2. Metatarsal: from metatarsal network on dorsal face (using a syringe with 22G needle, 0.7 x 30 mm). This is a useful technique to take blood with the animal into the water, because the fin cannot be straight.
3. Tarsal: from tarsal sinus on ventral face (using a Vacutainer System with 20G needle, 0.9 x 30 mm). Taking blood from a sinus, this is a useful technique because it allows having a pretty amount in a few minutes. The fin must be straight with respect to the body of the animal, so this technique is better with the animal out of the water.
4. Extradural: from extradural vein in the epidural sinus (using a syringe with 20G needle, 0.9 x 70 mm). This is a very good method to take blood with the animal out of the water and in straight position. It is also useful to administer fluids.

ANIMAL WELFARE HUSBANDRY: THE USE OF OPERANT CONDITIONING IN THREE AUSTRALIAN PELICANS (*PELECANUS CONSPICILLATUS*)

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Since the summer of 2012 Zoomarine Italy hosts a young group of three Australian pelican (*Pelecanus conspicillatus*) females in a big and mixed species exhibit with other aquatic birds. Thanks to training of medical behaviours it is possible to ensure a high standard of animal welfare monitoring. By using operant conditioning based on positive reinforcement, birds can be trained to participate voluntarily in welfare behaviours hence avoiding or minimising stress. The relationship and trust that can be achieved with the animals under human care is the basis for successful training. The training protocol consists of: desensitising; changing food habits; targeting; stationing; moving from A to B.

These behaviours are considered basic husbandry training finalised to facilitate daily animals' management. The most important application of this training method is in preventative medicine. Many wild species hide their symptoms making such training essential. By application of this training protocol, we obtain voluntary following behaviours: weighing; general physical examination; specific feet check thanks to a handmade pedometer; specific wing check.

Thanks to this successful training programme we have improved the quality of management of our pelicans and in addition we have included these results in an educational presentation for our public. This type of interactive session with zoo visitors helps to promote important issues about conservation and biodiversity.

POSITIVE REINFORCEMENT TRAINING OF A BOTTLENOSE DOLPHIN (*TURSIOPS TRUNCATUS*) CALF TO FACILITATE BLOOD SAMPLING

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By using operant conditioning based on positive reinforcement, the animals under human care can be trained to participate voluntarily in welfare behaviours hence avoiding or minimising stress. Welfare behaviours are considered the basic husbandry training finalised to facilitate the daily animal management. The relationship and the trust between animals and trainers is the basis of a successful training. Thai is the first calf of bottlenose dolphin (*Tursiops truncatus*) born at Zoomarine in September 2012. From the beginning, Thai was used to be in contact with the trainers due to the excellent relationship and trust that Leah, her mother, had with the trainers. Training sessions had a variable programme: interaction into the water, play and other usual activities. By increasing of interactive sessions, Thai started looking for the contact with the trainers more often. Our biggest challenge was to maintain this behaviour using relationship as the only reinforcement. In fact, in that moment Thai was still suckling and had just started eating fish. Thanks to a constant training programme, Thai was just one year old when the first successful blood sample without any stressful event or sanction was taken. Nowadays, his behaviour is very stable and positive.

TREATMENT OF A WOUND IN A BOTTLENOSE DOLPHIN (*TURSIOPS TRUNCATUS*) CALF USING A NEW WOUND-HEALING STIMULANT

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This case report describes the medical treatment of a soft tissue wound located at the ventral surface of the rostrum of a one year old bottlenose dolphin (*Tursiops truncatus*). The calf was hosted at Zoomarine Italia. The animal was found by the trainers with a flap of hanging skin, 4.5 x 2.5 cm. The wound was immediately rinsed and cleaned, and the hanging flap of skin was cut. No systemic therapy was done because the calf had a good attitude; also blood results were in the range for the species on laboratory evaluation. A recently developed wound-healing stimulant in an oily form, Neem tree (*Azadirachta indica*) and St John's-wort extract (*Hypericum perforatum*) (Hypermix Vet[®], RI.MOS. srl, Mirandola, Italy), was applied during the behavioural training twice a day. Hypermix Vet[®], so far never used in dolphins in accordance with the literature, is a product already effectively used in other domestic and exotic species for some years. After one month of therapy the wound was completely healed. General health status, management and nutrition of the patient are very important in wound healing. In the presented case we achieved good results by using a wound-healing stimulant based on Neem tree and *Hypericum perforatum* extract, but more experience is needed in order to prove the general benefit of this treatment.

PULMONARY CARCINOSARCOMA IN A CALIFORNIAN SEA LION (*ZALOPHUS CALIFORNIANUS*) KEPT UNDER HUMAN CARE

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A 15 year old intact male Californian sea lion (*Zalophus californianus*) with a previously unremarkable medical history became progressively lethargic and anorexic and developed severe respiratory distress and difficulty swallowing. Complete blood count and biochemistry profile were normal. Symptomatic therapy with antibiotic and anti-inflammatory drugs for 10 days was unsuccessful and the animal maintained its neck hyperextended for breathing. On day 12 sedation was attempted for X-rays and bronchoscopy, but the animal died under anaesthesia. At necropsy, 38 l of sero-fibrinous fluid was found in the thoracic cavity and both lungs were atelectatic. Multiple single or coalescent whitish and smooth nodules were observed on the pleura, pericardial sac, diaphragm, in mediastinal tissues, myocardium, muscles of the neck and laryngeal wall. Tracheo-bronchial and mesenteric lymph nodes were slightly enlarged. Samples were collected, formalin-fixed, paraffin-embedded and processed routinely for histologic examination. Haematoxylin eosin staining was performed on selected tissue sections. Prussian blue histochemical staining, electron microscopy and immunohistochemistry (IHC) were attempted on lung tissue and pleural masses in order to detect any asbestos particles. Histologically, tumour cells were poorly organised into elongated fascicles with an interwoven (herringbone) pattern. The tumour infiltrated massively the parietal and visceral pleura and the lung parenchyma focally and had a high infiltrative pattern. Tumour cells were small, generally spindle-shaped, with very scanty basophilic cytoplasm and indistinct cell borders, nuclei were plump, vesicular and elongated with indistinct nucleoli. In sections obtained from the pleural and pericardial nodules, anisokaryosis and anisocytosis were moderate. IHC on sections from pleural masses, lung and lymph nodes was performed using PulmoPanel™ Multiplex IHC (Biocare Medical, Milan, Italy). IHC confirmed the origin or sarcomatous differentiation of the tumour (vimentin positive and panCK positive). The PAS staining and PASD showed no accumulation of material (or mucins, or glycogen). Calretinin was negative both in the neoplastic elements that in the mesothelium. No evidence of pulmonary osteoarthropathia / akropachia was observed. These exams showed the presence of a pulmonary carcinosarcoma with widespread metastases. The lack of data regarding the populations in nature and the impossibility of subjecting all carcasses necropsied led to believe that the incidence of neoplasm in pinnipeds was low. However, California sea lions are an exception and in wild population tumours have been associated with herpesvirus, environmental contaminants and immunogenetic factors.

INTESTINAL T-CELL LYMPHOMA IN A HARBOUR SEAL (*PHOCA VITULINA*)

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Summary

A 14 year old harbour seal (*Phoca vitulina*) exhibited fatigue, anorexia, diarrhoea, and weight loss. Ultrasound, X-ray and computed tomography examination led to the suspicion of an abdominal neoplastic disease. After euthanasia, *post mortem* examination revealed an intestinal T-cell lymphoma. This is the first report of an intestinal T-cell lymphoma in a harbour seal.

Introduction

In captive and free ranging pinnipeds, tumours of various tissues are increasingly diagnosed as a cause of disease and / or death (NEWMAN and SMITH, 2006). There are few reports about neoplastic lymphoproliferative disorders in pinnipeds: a malignant lymphoma in an infant northern fur seal (*Callorhinus ursinus*) (STEDHAM *et al.*, 1977), a malignant lymphoma in a harbour seal (STROUD and STEVENS, 1980) a meningeal T-cell-lymphoma in a harbour seal (LABRUT *et al.*, 2007) and an intestinal T-cell-lymphoma in a California sea lion (*Zalophus californianus*) (COLEGROVE *et al.*, 2010).

Material and methods

The 14 year old male harbour seal was examined clinically. Laboratory investigations included blood cell count, serum biochemistry, faecal parasitology and bacteriology. Because the symptoms reoccurred, X-ray, ultra-sonography and computed tomography (CT) were performed under general anaesthesia. A necropsy was performed, including histology and immunohistochemistry.

Results and discussion

The harbour seal inconsistently exhibited signs of fatigue, anorexia, diarrhoea and reduced body condition. Three other seals in the same enclosure did not show any signs of disease.

The general examination of the patient did not reveal any abnormalities except the above symptoms.

Laboratory findings revealed mild anaemia, moderate hypoproteinaemia and hypoglobulinaemia as well as moderately elevated blood urea nitrogen. A reduced and compromised uptake of nutrients from the intestine was suspected.

Additionally, serum alpha-fetoprotein was investigated, which is known as a biomarker for lymphoma in dogs (LECHOWSKI *et al.*, 2002; WITHROW *et al.*, 2013). The result did not exceed the reference interval for domestic dogs.

Faecal parasitology and microbiology did not reveal any pathogens.

X-ray examination of the abdomen showed a severe loss of contrast due to depletion of the abdominal fat. Ultrasound examination revealed a rod shaped mass in the dorso-cranial abdomen, which was confirmed by CT. A neoplastic disease was suspected. The clinical findings and the general state of the animal led to the decision to euthanise the animal.

When euthanised the patient weighed 58 kg while another male from the same enclosure of equal length and age weighed 105 kg.

Gross findings during necropsy included emaciation, severe enlargement of the mesenteric lymph node and a mild erosive gastritis.

Histology revealed moderate to severe infiltration of blastoid cells in the mesenteric and inguinal lymph node and the intestinal mucosa. A purulent lymphadenitis was present only in the mesenteric lymph node. The small intestinal mucosa was infiltrated by small lymphocytes, single neutrophilic granulocytes, some blastoid cells and single plasma cells. A minor chronic focal non-purulent interstitial nephritis correlated with the elevated blood urea nitrogen.

Immunohistological findings in the mesenteric lymph node showed almost all T lymphocytes to be positive for CD3, several for CD79alpha and up to ten percent positive for MUM1. The axillary and inguinal lymph nodes contained up to seventy percent CD3 positive lymphocytes within the cortex and nests of MUM1 positive cells. The lymphocytes infiltrating the intestinal mucosa were nearly all positive for CD3; CD79alpha positive cells were only found within lymphatic follicles.

The clinical and pathological findings led to the diagnosis of an intestinal T-cell-lymphoma with metastasis in the mesenteric lymph node. The only intestinal T-cell lymphoma in a pinniped that has been described so far occurred in a California sea lion (COLEGROVE *et al.*, 2010). Intestinal malignant lymphoma should be considered as differential diagnosis for gastro-intestinal symptoms in harbour seals. Reference intervals for this biomarker are not known for pinniped species.

Biomarkers for neoplastic diseases like alpha-fetoprotein are not yet an established diagnostic tool for neither pinnipeds nor zoo and wild animals in general. The closer look at this fairly non-invasive diagnostic method in zoo and wild animals appears highly desirable, not only for the diagnosis, but also for the monitoring of therapeutic attempts, as shown in dogs receiving chemotherapy for lymphomas (LECHOWSKI *et al.*, 2002).

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HUMAN QUARANTINE AS A PREVENTIVE MEASURE TO PROTECT WILD HABITUATED GREAT APES FROM ANTHROPOZOONOTIC RESPIRATORY INFECTION

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Due to their close genetic proximity great apes are susceptible to several common human pathogens. These pathogens include respiratory viruses, such as respiratory syncytial virus (RSV) and human metapneumovirus (HMPV), as well as bacteria, like *Streptococcus pneumoniae*. Even though in healthy human adults these pathogens generally do not cause severe disease, infections in great apes have been shown to have dreadful outcomes with high morbidity and considerable mortality. Exposure to human pathogens can occur, for instance, when great apes are habituated to human presence, for research or tourism. However, nothing is known about the frequency of exposure to human respiratory pathogens or the infectious pressure in projects working with wild habituated great apes.

In recent years, many projects have improved their health management and implemented hygiene rules. These rules include overall limited numbers of people with controlled health status entering great ape habitat, the wearing of surgical masks or respirators, minimum viewing distance of seven metres, a ban on smoking, eating, drinking or spitting in the forest, removal of all human faeces, separate sets of clothes for camp and forest and personal hygiene, such as washing and disinfecting hands.

Some projects, like the Tai Chimpanzee Project of the Max Planck Institute for Evolutionary Anthropology and Karisoke Research Centre in Rwanda, have taken preventive measures a step further and implemented quarantine for humans intending to visit the great apes. Often people who come to see the apes have travelled from overseas and might have had exposure to a variety of pathogens throughout their travel, including in airports and airplanes. Additionally, stress of travel and change in climate increases the likelihood of getting infected, with respiratory pathogens being easily transmitted via airborne or fomite route. However, local project staff can also pose an increased risk when being in frequent contact with children in their villages.

RSV has an incubation period, which ranges from two to eight days, and HMPV between three and nine days. With a five-day quarantine, for instance, an important part of this period is covered and the chances of a person developing symptoms and shedding pathogens in the forest are decreased. Unfortunately, quarantine is very time- and therefore cost-intensive, requires a certain minimum of infrastructure (separate buildings and facilities) and can be complex to manage. We discuss the possibility and feasibility of implementing quarantine in habituation projects as a cost-intense but possibly necessary preventive measure to protect endangered great apes from human infection.

BLOOD PARASITES IN MALAGASY LEMURS (*AVAHI OCCIDENTALIS* AND *LEPILEMUR EDWARDSI*): THE EFFECT OF SLEEPING SITES AND SEASON

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The Milne-Edward's sportive lemur (*Lepilemur edwardsi*) and the Western woolly lemur (*Avahi occidentalis*) are weasel-sized Malagasy primates. Both species inhabit dry deciduous forests with prominent seasonal changes in climate and vegetation between the dry and rainy season. Furthermore they are nocturnal, monogamous, folivorous, and have a comparable body size but differ in their choice of sleeping sites. 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 (RASOLOHARIJAONA *et al.*, 2003, RAMANANKIRAHINA *et al.*, 2012). We expected that sleeping site ecology affects blood helminths: 26 free ranging sportive lemurs and 24 free ranging woolly lemurs were captured during the dry and the rainy season in the National Park of Ankarafantsika in north western Madagascar. Six couples of each species were radio collared for observation of their sleeping site behaviour and blood samples were taken. The sleeping site habits described in previous studies could be confirmed in so far as sportive lemurs had only up to five different sleeping sites within their home range which they change less often than the woolly lemurs with up to 31 sleeping sites. First data suggest that more than half of the individuals ($n = 32$) in both species show microfilaria in the Giemsa stained blood smears. The findings were verified by PCR. Sanger sequencing could not assign the microfilaria to a genus. Further investigation on molecular level is still in progress.

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FISH SKIN LESIONS: AN OLD PROBLEM ANALYSED IN A NEW WAY. COMPARATIVE CLINICAL CASES

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In fish medicine, skin lesions are very common and their aetiology and pathogenesis are widely described in literature. Over the last few years atypical lesions are becoming increasingly common in veterinary clinical practice and these lesions often results to be difficult to classify. New diagnostic tools and modern laboratory techniques such as ultrasound, endoscopy and PCR have changed the clinical approach to fish diseases and they have provided valuable assistance to the clinician veterinarian. Problem approach and therapeutic choices also changed since new methodologies, such as electrocautery, have been successfully used with aquatic animals. Eight different clinical cases of fish skin diseases have been compared and analysed.

Case 1: Goldfish (*Carassius auratus*) with an atypical cutaneous lesion and no other internal lesions. Molecular probe identified *Mycobacterium marinum*.

Case 2: Goldfish (*Carassius auratus*) with cysts at the caudal peduncle. *Aeromonas* spp. was diagnosed using microbiological swab. Two month later echography was performed since the fish was found lying on the bottom. A kidney mass was identified and the animal euthanised. PCR detected *Mycobacterium* spp.

Case 3: Goldfish (*Carassius auratus*) with an ulcerated and haemorrhagic mass. Diagnosis was performed by biopsy, infiltrative fibrosarcoma poorly differentiated with low metastatic potential was diagnosed and electrosurgical excision was chosen.

Case 4: Koi carp (*Cyprinus carpio*) with multiple body masses. Microbiological swabs were negative. Ecography showed a large mass in coelom cavity at the kidneys. After euthanasia an infiltrative nephroblastoma was diagnosed.

Case 5: Koi carp (*Cyprinus carpio*) with a large tumefaction. Bacteriology identified *Citrobacter freundii*, antibiotic therapy was administered but the animal died after 15 days. Necropsy revealed a fistula corresponding to the prophylactic antibiotic injection administered after the transport two month before the problem onset.

Case 6: Mediterranean moray (*Muraena Helena*) with 2 - 150 mm multiple cysts. Microbiological swabs were negative. Necropsy showed a systemic mycosis and an oesophagus rabiomioma.

Case 7: Goldfish (*Carassius auratus*) presented with cutaneous ecchymosis. CT scan revealed a mass at the kidenys. Filter slime filter was sampled to run molecular probes and *Mycobacterium marinum* was isolated.

Case 8: Stellate puffer (*Arothron stellatus*) with gill operculum mass compatible with abscess. Microbiology was negative. The animal was moved to a bigger tank equipped with an effectiveness biological filter. Mass regressed to disappear in three days.

This work aims to describe different practical problems, possible diagnostic approaches, mistakes and elective therapeutic choices encountered, comparing the results obtained with the data reported in literature.

SEROLOGICAL STUDY OF INFECTIOUS AGENTS OF WILD NON-HUMAN PRIMATES IN COSTA RICA

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The objective of the study was to detect the presence of antibodies against *Mycobacterium avium* subspecies *paratuberculosis* (MAP), *Neospora caninum*, Influenza A virus (IAV), West Nile Virus (WNV) E protein (prE), and against other viruses from the Japanese encephalitis virus (JEV) complex, in captive and wild non-human primates in Costa Rica.

During 2010 and 2012, 59 blood samples were processed and taken from wild non-human primates belonging to different regions, aiming to study the condition of primates in Costa Rica, the study included species such as *Alouatta palliata*, *Saimiri osterdii*, *Cebus capucinus*, and *Ateles geoffroyi*. The animals were anaesthetised with a ketamine-xylazine (Ketamin 10 %, 0.02 mg/kg, BREMER PHARMA GMBH, Warburg, Germany; Xylazine 2 %, 0.15 mg/kg, ALFASAN, Woerden, The Netherlands) mixture using a dart, and parameters were taken during the anaesthesia time. After recovery from the anaesthesia, they were released back in the same place they were captured. Physical examination was performed and the blood samples were sent to the School of Veterinary Medicine's laboratory of Entomology, where they were analysed to determine the presence of antibodies against West Nile virus (WNV), *Mycobacterium avium* subspecies *paratuberculosis* (MAP), *Neospora caninum*, and Influenza A virus (IAV), using competitive enzyme immunoassays. The most prevalent agent found was the WNV (32/66, 48.5 %), meanwhile two white-faced capuchins from Colorado, Pococí, Limón, and from San Joaquín, Barranca, Puntarenas, showed antibodies against MAP (1/58, 1.7 %) and *N. caninum* (1/50, 2 %), respectively. None of the 59 samples resulted positive for IAV. Animals with antibodies did not necessarily show clinical signs related to the presence of the agent.

It is important to conduct further studies in the regions where antibodies were detected in the animals of the same place, and determine a relationship with human populations and populations of other species of the same area.

WILDLIFE EMERGENCY CLASSIFICATION IN COSTA RICA

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In Costa Rica the increase of human development produce pressure on wild animals which suffer from emergencies as shown in table 1. The objective of this study was to know the emergencies in relation to the environmental awareness. We obtained information through many ways and analysed the management of the emergencies. The data collection method was based on different calls, taking care of the emergencies and information from the rescue centres.

Tab. 1: Wildlife emergency classifications in Costa Rica. Families: 1 Felidae, 2 Canidae, 3 Didelphidae, 4 Ramphostidae, 5 Psittacidae, 6 Cracidae, 7 Columbidae, 8 Passeridae, 9 Cebidae, 10 Dasyproctidae, 11 Atelidae, 12 Erethizontidae, 13 Tayassuidae, 14 Geomyidae, 15 Sciuridae, 16 Leporidae, 17 Cyclopedidae, 18 Bradypodidae, 19 Megalonychidae, 20 Cuniculidae, 21 Tapiridae, 22 Viperidae, 23 Procyonidae, 24 Dasypodidae, 25 Myrmecophagidae, 26 Dasyponidae, 27 Geomyidae, 28 Cathartidae, 29 Pelicanidae, 30 Ardeidae, 31 Turdidae, 32 Boidae, 33 Cervidae, 34 Mustelidae, and 35 Falconidae.

Case	Percentage (%)	(n)	Families involved *(family (n))
Electrocution	18	27	* 19(2), 18(4), 25(2), 11(4), 30(5), 29(2), 5(1), 23(2), 28(2), 9(3).
Trauma in highways	26	39	19(3), 18(5), 25(5), 20(2), 16(5), 30(4), 1(4), 10(1), 33(1), 13(3), 7(1), 3(2), 32(3), 22(2).
Animals entering in human landscapes	18	27	19(1), 25(1), 12(1), 5(6), 9(5), 14(2), 2(1), 3(2), 32(1), 6(1).
Traumatic injuries	14	21	18(2), 25(2), 22(3), 26(2), 20(1), 12(3), 15(2), 16(1), 9(3), 29(1), 14(2).
Intoxications	10	15	10(2), 27(3), 15(2), 16(2), 5(2), 29(3), 35(1).
Shock of unknown cause	6	9	17(1), 25(1), 20(1), 15(1), 16(1), 5(1), 9(2).
Orphans	8	12	1(2), 21(1), 18(2), 31(2), 8(2), 11(2), 4(1).
Death	14	21	13(1), 9(2), 33(1), 7(1), 5(2), 11(2), 29(1), 25(3), 22(3), 3(3), 28(2)

Results showed 150 cases with complete information during 2014. As shown in table 1, 18 % of the cases were found injured or dead due to electrocutions, 26 % traumas on highways, 18 % animals entering human landscapes, 14 % traumatic injuries, 8 % orphans, 10 % intoxications, and 6 % shock of unknown cause. Animals which did not get immediate veterinary attention died. This study showed 14 % mortality rate, including received animals from critical condition. This can be extended due to aspects such as budget shortage and the lack of employee training to transport animals. It is necessary to train government entities, educate them regarding emergency management and provide contacts to veterinary facilities in various regions. It is also recommended to work on wildlife crossing areas. Citizens need to be educated about humanitarian assistance of wildlife, and signs on wild animal crossing areas on roads are needed to reduce cases of collisions.

PROVENTRICULAR SPIRURIDIASIS AND PROLIFERATIVE DISEASE IN CAPTIVE PSITTACINE BIRDS: 14 CASES

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Fourteen cases of proventriculitis associated with nematodiasis in zoo and aviary psittacine birds were retrospectively evaluated. Represented species include rainbow lorikeets (*Trichoglossus haematodus*) (seven cases) and one case each of budgerigar (*Melopsittacus undulatus*), twenty-eight parrot (*Barnardius zonarius semitorquatus*), scarlet chested parrot (*Neophema splendida*), bluebonnet (*Northiella haematogaster*), red-rumped parrot (*Psephotus haematonotus*), dusky lory (*Pseudeos fuscata*), and gold and blue macaw (*Ara ararauna*). Affected birds presented with evidence of gastrointestinal disease including proventricular dilatation or were found dead with no premonitory signs. Grossly, thickening of the proventricular mucosa, proventricular dilatation, accumulation of excessive mucus in the proventriculus sometimes with nematodes, presence of whitish polypoid masses in the proventricular mucosa, dark red foci within proventricular glands, gastric bleeding, or a combination of several of these lesions were observed. Histopathological examination revealed severe diffuse proliferative to focal adenomatous proventriculitis with intralesional nematodes consistent with spirurids in all birds. Parasites were characterised by a cuticle with transverse striations, a polymyarian and coelomyarian musculature, a large intestine lined by columnar cells with a brush border, a gravid uterus with small, thick-shelled embryonated eggs, as well as eosinophilic material within the pseudocoelom. Cuticular cordons, which are consistent with acuariid spirurids, were often noted histologically on its anterior end, which was identifiable by the presence of tri-radiate esophagi. Some nematodes were embedded in the mucosa or in adenomatous polyps. In the bluebonnet and dusky lory, parasites were identified as *Dispharynx nasuta* and *D. spiralis*, respectively, by morphological evaluation of adult nematodes. An invasive spindle cell tumour with perivascular whorling consistent with a haemangiopericytoma was observed in the submucosa and muscular tunics of the proventriculus in the bluebonnet. The budgerigar and the red-rumped parrot also had *Macrorhabdus* associated with the proventricular lesions. Aspiration pneumonia and air sacculitis attributable to gastric disease were observed in one case. According to these findings and previous reports in other species, acuariid spirurid nematodes appear to be relevant pathogens in psittacine birds and are often associated with severe proliferative proventriculitis and proventricular adenomatous polyps. In one of the cases, a proventricular haemangiopericytoma was found underlying proventriculitis and has not been previously reported in proventricular spiruridiasis. In this regard, the spirurid *Spirocerca lupi* is known to cause sarcomas in the oesophagus of dogs. Rainbow lorikeets appear to be overrepresented in this study and therefore a possible species predisposition should be investigated. Proventricular spiruridiasis should be included in the differential diagnoses of proventricular dilatation and thickening, proventricular masses, and gastrointestinal bleeding in psittacine birds.

FATAL COLITIS IN JUVENILE COMMON ELANDS (*TAUROTRAGUS ORYX*) ASSOCIATED WITH CORONAVIRUS INFECTION

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Four captive juvenile common elands (*Taurotragus oryx*) died within nine days after presenting with diarrhoea at the beginning of winter 2011. All four animals were subjected to *post mortem* examination, which revealed serous atrophy of adipose tissue, muscular atrophy, faecal staining of perineum and hind limbs, and watery to pasty contents in the large intestine. Tissues (small and large intestine, abomasum, mesenteric lymph nodes, spleen, liver, lung, kidney, adipose tissue and/or heart) from two elands were submitted for histopathological examination. Microscopically, both elands had loss and necrosis of crypts in the colon. Crypts were often dilated and contained luminal accumulations of debris and necrotic enterocytes. In addition, both elands had severe serous atrophy of adipose tissue and lymphoid hyperplasia in mesenteric lymph nodes. Immunohistochemistry using mouse monoclonal antibodies against bovine coronavirus (RTI, LLC; Brookings, SD, USA) revealed abundant coronavirus antigen within the cytoplasm and less frequently in the nucleus of colonic enterocytes lining necrotic crypts and the surface of mucosa. Additionally, weaker staining was noted in enterocytes of the tip of villi in the ileum and macrophages in the mesenteric lymph nodes. BCoV are frequently involved in cattle, in cases of calf diarrhoea and winter dysentery (FULTON *et al.*, 2015). Viruses sharing over 99 % nucleotide identity with BCoV have been detected by PCR and sequencing in faeces and intestinal contents of a variety of wild ruminants in captivity, not including the common eland, often in cases of diarrhoea (ALEKSEEV *et al.*, 2008; CHUNG *et al.*, 2011). However, to the authors' knowledge, fatal colitis and intralesional identification of BCoV antigen by immunohistochemistry has not been described in zoo ruminants. This study adds elands to the list of susceptible zoo ruminant species. Location of lesions in the colon and involvement of crypts mimics descriptions of fatal BCoV infection in cattle (FULTON *et al.*, 2015). BCOVs or closely-related group 2a coronaviruses should be considered in the differential diagnosis of diarrhoea and fatal colitis in zoo ruminants including common elands.

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DISSEMINATED SPIRORCHIIDIASIS IN CAPTIVE EUROPEAN POND TURTLES (*EMYS ORBICULARIS*) CAUSING FATAL TREMATODE EGG THROMBOEMBOLISM AND INTESTINAL NECROSIS IN SPAIN

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Two captive adult European pond turtle (*Emys orbicularis*) died shortly after presentation with non-specific symptoms and cachexia in a wildlife rescue centre in Madrid (Spain) in 2008 and 2009. These captive turtles had been donated after unknown periods of time in captivity. They were housed in an artificial pond adjacent to Spanish pond turtle (*Mauremys leprosa*) enclosure and died four years (one case) or four months (one case) after admission at the centre. Gross examination of both turtles revealed severe thickening of the intestine due to full-thickness necrotising lesions, as well as multifocal or diffuse thickening with tortuosity of arteries in the intestinal mesos and walls; affected segments had a dark red colour. Histopathological examination revealed disseminated embolism of trematode eggs, particularly in the intestinal blood vessels, but also in the stomach, liver, kidneys, lungs, adrenal glands and other organs. Egg embolism in the intestinal vessels was often massive and associated with severe acute transmural necrosis of the intestinal wall. A bacterial component secondary to necrosis was observed. Thrombosis and granulomatous vasculitis were noted in some of the affected vessels. The intravascular location and morphology of parasites were consistent with spirorchiid trematodes. Spirorchiidiasis has been reported in sea and freshwater turtles (JOHNSON *et al.*, 1998; GORDON *et al.*, 1998). To the authors' knowledge, these appear to be the first documented cases of fatal disseminated spirorchiidiasis in captive European pond or other indigenous freshwater turtles in Europe. The source of this parasitism is unknown. Massive embolism of intestinal arteries with thrombosis and vasculitis likely caused local ischemia and is therefore suspected to have caused fatal necrosis of the intestine in these turtles.

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FELINE PANLEUKOPENIA IN SEVEN DIFFERENT CAPTIVE FELINE SPECIES IN THE UNITED ARAB EMIRATES

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Feline panleukopenia virus (FPV) is a species of the genus parvovirus. The virus is highly contagious and often fatal, especially in kittens. It is very resistant and can persist in the environment at room temperature for over one year. Virus particles are abundant in all secretions and excretions during the acute phase of illness and can be shed in the faeces of survivors for up to six weeks after recovery. Animals can be infected via oronasal transmission.

Feline panleukopenia has been detected in 14 animals belonging to seven different feline species in the United Arab Emirates in the last 10 years. Affected were six cheetahs (*Acinonyx jubatus*), three Arabian leopards (*Panthera pardus nimr*), one black-footed cat (*Felis nigripes*), one caracal (*Caracal caracal*), one jaguar (*Panthera onca*), one lion (*Panthera leo*) and one marbled cat (*Pardofelis marmorata*).

Most of the animals were older than one year and showed clinical signs like anorexia, diarrhoea and faintness. All animals showed marked to severe catarrhal to haemorrhagic enteritis, typical for parvovirus-enteritis. Histology revealed atrophic enteritis with numerous distended and some necrotic crypts, a few in regeneration stage, and marked depletion of Peyer's patches. No intranuclear inclusions were observed in intestinal crypt cells. However, most cases were affected by a certain degree of autolysis.

Twelve of these animals were tested positive for parvovirus via Rapid Immuno-Migration test (RIM™), two animals were tested negative. No vaccination history was available in most of the cases. However, since vaccination success is uncertain in nondomestic felids, FPV may also occur in vaccinated animals. As shown in our cases from seven different nondomestic felid species, any felid kept in captivity might be affected.

A NEWLY RECOGNISED FELINE INFECTIOUS PERITONITIS-LIKE DISEASE IN MINK (*MUSTELA VISON*) ASSOCIATED WITH AN ALPHACORONAVIRUS

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Coronaviruses have previously been associated with both enteric and systemic infections in several species. An FIP-like disease was recognised microscopically in seven mink. The objectives of this study were to 1) describe the gross and microscopic lesions associated with this FIP-like disease 2) use immunohistochemistry (IHC), RT-PCR and sequencing to define whether a coronavirus was associated with the lesions and 3) investigate the genetic relationship between this coronavirus and the previously described mink coronavirus (MCoV) associated with enzootic catarrhal gastroenteritis (ECG).

Seven mink (*Mustela vison*), from three months to one year of age, were included in this study. Gross lesions were reported for only one mink as multifocal coalescing firm, white nodules within the pancreas and the mesentery. Microscopically, the pancreas was characterised by multifocal regions of pyogranulomatous inflammation, necrosis, and fibrosis. Additionally, this mink also had moderate pyogranulomatous meningitis, myocarditis, hepatitis, and necrotising splenitis. The remaining six animals were selected from archival paraffin blocks, based on the report of pyogranulomatous lesions of unknown cause. Microscopically, all mink had multifocal regions of pyogranulomatous inflammation involving one or more organs, including pancreas, meninges, heart, spleen, abdominal lymph nodes, liver, and/or mesenteric tissues. Alphacoronavirus antigen was detected by IHC in macrophages within pyogranulomatous lesions from all mink. IHC was performed using a mouse monoclonal antibody against alphacoronaviruses (FIPV3-70, Custom Monoclonals International, Sacramento, CA, USA) as the primary antibody and an alkaline phosphatase detection system with a red chromogen on the Ventana Discovery Ultra automated staining system.

Generic coronavirus RT-PCR that targets a 179-bp region of open reading frame 1b (ORF1b) was performed on RNA extracted from frozen tissues available for one mink. The sample was found positive for coronavirus RNA. Subsequent RT-PCR and sequencing of a portion of the spike gene yielded a unique 651 bp sequence that was analysed by BLAST against the GenBank database. The newly identified sequence was 89 % identical with the analogous sequence of ECG-associated MCoV. BLAST analysis also revealed a significant similarity of the mink-derived sequence to equivalent porcine, canine and feline coronavirus sequences, at ~ 73 % identity. Corresponding published sequences of ferret coronaviruses showed ~ 70 % similarity to the mink sequence.

A previously unrecognised FIP-like disease was identified in mink. The combined IHC, RT-PCR and sequencing data confirmed an alphacoronavirus associated with the lesions. Sequencing of a portion of the S gene indicated that this virus is genetically most closely related to MCoV. Whether there is consistent genetic fingerprint associated with this novel mink coronavirus that is different from the previously recognised ECG-associated mink coronavirus, needs to be further investigated.

HUSBANDRY OF TWO SPECIES OF FLYING FOXES (*EIDOLON HELVUM* AND *ROUSETTUS AEGYPTIACUS*) IN A LABORATORY ANIMAL SETTING

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Bats (*Chiroptera*) play an important and significant part within the global ecosystems. In recent years, various bat populations worldwide have decreased in numbers due to anthropogenic habitat changes, disease outbreaks and purposive killing of bats by humans as prey or pests. At the same time, current outbreaks in humans and new research results emphasise their role in the transmission of highly infectious zoonotic diseases such as Ebola, Nipah, Rabies or SARS. To prevent and decrease the risk of bat-related disease outbreaks in human populations and improve the conservation of bats and their native environment, research on the role of bats in the epidemiology and infectiology of pathogens has to be intensified. For this purpose, the Institute of Novel and Emerging Infectious Diseases (INEID) has established breeding groups of two species of African flying foxes (*Eidolon helvum* and *Rousettus aegyptiacus*) on the grounds of the laboratory animal holding facilities of the FLI Riems.

Both species are kept separately in two L-shaped indoor aviaries of 22 qm² floor space and 2.5 m height. Temperatures and humidity are kept at 24 – 26°C and 60 % respectively. An automatic 12 h artificial circadian light regime including UV-B lighting replaces the absent sunlight. The enclosures are cleaned at least once daily. The animals are provided with a great variety of fruits and vegetables as well as varying arrays of behavioural enrichment elements. Special care is undertaken in regard to limiting the feeding of Vitamin C-rich food in particular to *Rousettus aegyptiacus* to decrease the risk of haemochromatosis.

The veterinary care part consists of regular health screening, including sampling for bacteriology, parasitology and virology both in the quarantine and in the main husbandry department, as well as the treatment and abatement of acute and chronic medical issues, such as injuries related to intraspecific aggression or age-related health problems. In general, both populations appear to be in good health, necessitating only infrequent medical intervention. At the moment, a medical training system has been started to facilitate regular health checks for both the staff and the animals. This training includes training practices developed at Animal Kingdom, Columbus Zoo and Lubee Bat Conservancy (all three: USA). As the next step, the current husbandry with focus on breeding will be augmented to include husbandries for laboratory trials, including infectiological experiments with viral pathogens. The establishment of cell lines derived from specimens of the collection has already been undertaken, as well as international collaboration with fellow institutions for the sake of providing them with sample material.

PREVALENCE OF SELECTED ZONOTIC PATHOGENS IN THE POPULATION OF WILD BIRDS IN AUSTRIA

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To determine the importance of wild birds as a reservoir of zoonotic pathogens in Austria, 763 wild birds were sampled. The majority of the birds belonged to the order *Passeriformes* (34 %), *Columbiformes* (34 %) and to birds of prey - *Accipitriformes*, *Strigiformes* and *Falconiformes* (17 %). From each bird two swabs, a cloacal swab (eSwab™, Copan, Brescia, Italy) for bacterial culture and a triple swab (conjunctiva, choana, cloaca) for DNA- and RNA-isolation (UTM™ swab, Copan, Brescia, Italy) were taken. The cloacal swabs were further processed by microbiological methods for *E. coli*, *Salmonella* spp., methicillin resistant *Staphylococcus aureus* and thermophilic *Campylobacter* spp.; nucleic acids isolated from the triple swabs served as template for PCR to detect West Nile Virus, Avian Influenza Virus and *Chlamydiaceae* spp. Further on, fresh droppings from 66 birds were cultured for *Cryptococcus* spp. and from 115 fresh carcasses tissue samples were investigated for the presence of *Mycobacterium* spp. by different PCR methods. The zoonotic bacteria most often detected proved to be *Campylobacter* spp. (12.3 %), followed by *Chlamydiaceae* spp. (9 %). 72.5 % of the sampled birds were positive for *E. coli*; PCR analyses revealed the intimin gene in 10 % of the *E. coli* samples and 0.42 % were positive for *stx1* and *stx2* genes. *Salmonella* spp. was rarely found in the sampled birds (0.8 %), similar to methicillin resistant *Staphylococcus aureus* (0.2 %). None of the samples were positive for *Cryptococcus neoformans*, *Mycobacterium* spp., Avian Influenza Virus or West Nile Virus. Further analyses of the *Campylobacter* spp. isolates found the majority to be *C. jejuni*, with the highest prevalence in the hooded crow (*Corvus cornix*). Concerning the prevalence of *C. jejuni* and the *Chlamydiaceae* spp. in the tested population, Austrian wild birds may be a source of these diseases implying a risk of infection especially for humans in close contact with them.

BATS AS VETERINARY PATIENTS

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All bat species and their roosts in the European Union are legally protected, by both domestic and international legislation. The protection of bats and their environment is also regulated by international agreements (Bonn Convention, EUROBATS Agreement and the Berne Convention). This conservation status makes bats an important object of veterinary medical care.

Bats can be found in all types of environment including human settlements. Many synanthropic bat species roost inside of human buildings year round. In Central European region individual bats occasionally enter houses, most often during summer evenings in mid-July and August. These gone astray bats are usually pups that are just beginning to fly. Individual bats may enter houses during the night hunting insects attracted by interior lighting. Fortunately, these incidents can be dealt with quite easily. Large hibernating aggregations and behaviourally triggered „autumn invasions“ of bats are known in European bats. Therefore the cases of finding of bats in the proximity of human are not uncommon. Animals may be exhausted, injured, dehydrated etc. Nowadays each veterinary practitioner and vet clinic should be prepared for attending bat patients.

Since 2009 we have rescued 756 bats belonging to five species. This paper summarises our experiences with veterinary care, especially first aid, fluid and medical therapy and complex rehabilitation of bats.

Dehydration is the most frequent complication in bats found inside buildings. Bats showing symptoms of dehydration and cachexia were treated with the combination of subcutaneous rehydration solutions Infusio Ringeri[®] (IMUNA, Šarišské Michaľany, Slovakia) and DUPHALYTE[®] A. U. V. INJ. (Pfizer Olot, Vall de Byania, Spain). The administration of 0.01 ml of DUPHALYTE[®] per each g of bats' weight showed the best results. Bats were fed with meal worms coated with the premix Roboran[®] H plv. (UNIVIT, Olomouc, Czech Republic). The dosage of whole rehydration solution was 0.15 ml pro toto each 8 h in small bats (pipistrelle bats) and 0.5 ml pro toto each 8 h in bigger bats (noctule bats, particoloured bats).

We recorded five cases of bats attacked by dogs or cats. These cases were associated with traumatic lesions. After cleaning and wound disinfection, systemic antibiotics were administered subcutaneously: Baytril 25 mg/ml inj. (KVP Pharma + Veterinär Produkte GmbH, Kiel, Germany) and Amoxicillin Bioveta 150 mg/ml LA (Bioveta, Ivanovice na Hané, Czech Republic) were used.

We have successfully used the method of induced artificial hibernation on bats rescued during the hibernation period. After seven days lasting hibernation in the refrigerator, animals were fed for two to three days and then returned into the hibernation box.

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ANTIMICROBIAL SUSCEPTIBILITY AND GENOTYPIC CHARACTERISTICS OF *CAMPYLOBACTER* SPP. ISOLATES FROM FREE-LIVING BIRDS IN POLAND

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Campylobacter (*C.*) spp. (*C. jejuni* principally) is the most commonly reported bacterial cause of human foodborne infection in European Union and United States of America. Commercial poultry and free-living birds are natural reservoirs of particularly three species: *C. jejuni*, *C. coli*, and *C. lari*. In addition, human *Campylobacter* isolates show increasing resistance to antimicrobial therapy. Little information is available on antimicrobial susceptibility of *Campylobacter* spp. isolated from free-living birds. The aim of this study was to examine and then determine the antimicrobial susceptibility of 43 *Campylobacter* strains obtained from intestines of seven free-living bird species in Poland. The genotypic characteristic of *Campylobacter* strains was performed using the PCR method described by WANGROONGSARB *et al.* (2011). The affiliation to the genus and *Campylobacter* species of isolates were determined.

In total 700 of birds were examined. The 43 positive samples were obtained from: mallards (29), great cormorants (5), velvet scoters (4), tawny owls (2), common buzzard (1), rook (1) and Eurasian tree sparrow (1). Thirty seven (86 %) strains belonged to *C. jejuni*, five (11.6 %) to *C. coli* (all isolated from great cormorants) and one (2.32 %) to *C. lari* (isolated from scoter).

The antimicrobial susceptibility was determined to every isolated *Campylobacter* strain using commercial Sensititre™ *Campylobacter* MIC plates (Thermo Scientific, Tewksbury, MA, USA) for nine antimicrobial substances (azithromycin, ciprofloxacin, erythromycin, gentamicin, tetracycline, florfenicol, nalidixic acid, telithromycin and clindamycin).

Sixteen of the examined strains (37.2 % of all positive samples) showed susceptibility to all of the nine antimicrobial substances. Twenty two (51.1 %) of examined strains were nalidixic acid resistant, seventeen (39.5 %) strains were resistant to ciprofloxacin and clindamycin as well. Moreover MIC values of ciprofloxacin susceptibility for next thirteen (30.2 %) strains amounted 0.12 µg/ml, what comprises a borderline-susceptibility according to the CLSI norms. Every strain that obtained a MIC value over 12 µg/ml was recognised as ciprofloxacin resistant strain. Thirteen (30.2 %) strains were tetracycline resistant. Nine strains (20.9 %) were erythromycin and florfenicol resistant. Seven (16.3 %) strains were gentamicin resistant, four (9.3 %) strains were resistant to telithromycin and only two (4.6 %) strains were azithromycin resistant.

Results obtained in this study suggest that special attention should be given to free-living birds and potential approaches for the control of antimicrobial-resistant *Campylobacter* should be discussed.

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RAISING NYOTA - EXPERIENCES OF SEMI HAND REARING AN OKAPI (OKAPIA JOHNSTONI)

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On the 6th of June 2012 a female okapi calf, named NYOTA (GAN HJZ12-00157) was born to KABINDA (GAN: 10697378). The first days after parturition were uneventful and Nyota drank colostrum. Kabinda was found dead on the 23rd of June. She had not displayed any clinical signs prior to death and necropsy revealed acute kidney failure (nephropathy and acute degeneration of the tubuli).

At that time a second breeding female, IBINA (GAN: MIG12-29801524), was leading a calf aged 6.5 months (*6th Dec. 2011). It was discussed and decided together with the curator, the EEP, the keepers and the vets to try to use her as a foster mother. A combination of hand and foster rearing was thought to be most beneficial for Nyota. She was thus introduced to Ibina one day after Kabinda's death. Of course it was important that the rearing of Ibina's calf was not jeopardised. Ibina accepted Nyota, but started to decrease in body condition due to the extended period of lactating and thus supplementary feedings were offered to her. Ibina's calf developed just fine.

The first two days after Kabinda's death we opted for a cow milk replacer (two days only), and then fresh cow milk was fed for almost two months. In the following months she received a self-developed okapi milk replacer, which was formulated to best match the values obtained from original okapi milk. This was possible because we were able to take a milk sample from the carcass of Kabinda and analyse it for its dietary composition. In the following days and weeks we also sent milk samples from Ibina in for analysis ($n = 15$). In general it can be stated that the okapi milk was composed of: 75 g/100 g water, 10.52 g/100 g protein and 8.36 g/100 g fat (mean values, Ibina's milk), though the fat levels of Kabinda's milk were higher and the protein levels were lower (74.6 g/100 g water; 9.3 g/100 g protein and 10.2 g/100 g fat, respectively). The mean values are also in compliance with the ones published. In that study the samples ($n = 17$) were analysed during the first six months of lactation in a captive held okapi. Deviating from that study the values of protein in our case did not increase during the course of the lactation, but we only started regularly sampling Ibina when her calf was already six months of age and therefore at a later stage of lactation. The results of the okapi milk analyses were compared to 'regular' cow milk. To adjust the cow to the okapi milk we thus added Aptamil protein plus[®] and Basic-ch[®] (both Milupa GmbH, Friedrichsdorf, Germany) to ensure the higher fat and protein levels needed. We continued this for about two months with ~ four feedings/day with a total of up to 1200 ml milk until Nyota did not accept the bottle feeding anymore and was weaned within a week by early November. She had defecated for the first time in August and had started eating solids from mid-October. The weight development of Nyota was closely monitored and today Nyota weighs a good 250 kg. Nyota will turn three this summer and may be paired with a breeding male next year.

The authors want to thank the okapi keepers for all their efforts and extra hours, the staff of the CVUA Stuttgart for the milk analyses (even the smallest amounts). Last but not least we want to thank the staff of Milupa GmbH, esp. Mrs. Müller, for helping us calculate the best formula to ensure Nyota's good growth and development.

References can be obtained from the author.

FIVE-YEAR SURVEY OF THE INTESTINAL PARASITES IN REPTILES, RATITES, HOOFED MAMMALS, CARNIVORES AND PRIMATES IN ZOO LJUBLJANA

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Summary

A survey was undertaken to investigate the prevalence of intestinal parasites from different groups of animals in Zoo Ljubljana, Slovenia. In five year period (2008 - 2012), a total of 350 faecal samples of 39 animal species were collected randomly and examined microscopically. The total prevalence of intestinal parasites was 42.6 % with the highest prevalence in hoofed mammals (54.6 %), then in reptiles (40.2 %), ratites (32 %) and primates (20 %); faeces of carnivores were negative. There were differences between groups of animals and years of survey. Parasites were detected in 18.8 - 87.5 % species of hoofed mammals, 20 - 60 % species of reptiles, 0 - 40 % species of primates and 0 - 66.6 % species of ratites during individual years. This is the first parasitological study in zoo animals in Slovenia. Routine monitoring of parasitic infection in zoo animals is important part of zoo management as preventive and control measures to minimise the spread of infectious parasitic diseases among animals within the zoo.

Introduction

Both domestic and wild animals are hosts to a wide variety of parasite species. Problems of parasitic infections and interspecies transmissions are also common in Zoos and could pose serious health damage to animals (COMBES, 2001; Lim *et al.*, 2008; ROBERTS *et al.*, 2009). Thanks to continuing efforts of parasitologists around the world, the identification and life cycles of many parasites are now well known. This knowledge, help us in diagnosing and treating such infections and use effective methods for the prevention and control of infection.

This study is dealing with the parasite diversity and prevalence of infection in animals kept in Zoo Ljubljana between years 2008 and 2012. The aim of this study is to understand the parasite dynamics and to evaluate the results and effectiveness of regular control of the gastrointestinal parasites in animals. The results should improve the prevention of infectious parasitic diseases among animals within the zoo.

Material and methods

Animals

This study took place in Zoo Ljubljana, which is located on the outskirts of Ljubljana, Slovenia. It covers an area of 6 ha and houses for about 544 animals of 125 different species. In total, 350 faecal

samples from animals of 39 different species (table 1) were examined. Among hoofed mammals, there were included domestic animals (donkey, goat, sheep and cow), autochthonous (Alpine ibex, European mouflon, chamois, red deer and fallow deer) and allochthonous (red lychee antelope, Chapman's zebra, alpaca, guanaco, bactrian camel, reticulated and Rothschild giraffe) mammals. The group of reptiles included nose-horned viper, four-lined snake, red corn snake, Indian python, royal python, yellow rat snake, bearded dragon, green iguana, bengal monitor and basking glass lizard. There were also ratites (ostrich, emu and rhea), carnivores (African lion, Persian leopard, brown bear, grey wolf, European badger) and primates (chimpanzee, squirrel monkey, black and white ruffed lemur, yellow cheeked gibbon, common marmoset) included in study.

Parasite monitoring

Each group of animals was repeatedly examined for parasites during years 2008 - 2012. In first two years of our survey, all animals were examined usually twice a year; in years 2010 and 2011 hoofed mammals were examined four times a year, the other twice a year and in the last year, all species, except reptiles were examined four times a year (March, June, September and November). Reptiles were examined only twice a year because of hibernation. In case of parasitic infection, the animals were treated and examined repeatedly to see efficiency of antiparasitic treatment. All animals coming to the Zoo, except of Balai Directive 92/65/EEC approved specimen, were kept in the quarantine at least for 30 days and examined for parasites using coproscopy, sporulation and larvoscopy to exclude possible introduction of parasites.

Coprological examination

Fresh faecal samples were collected randomly during the whole day from the floor in the enclosure. Mixed faecal samples, at least 5 grams, were collected from the whole group of animals of one species kept together. Samples were collected into the plastic vials and stored at 5°C for maximum two days before analysis. Modified Sheather's sugar solution (~ 1.27) was used for flotation (BLAGBURN and BUTLER, 2006). In case of suspected coccidiosis, samples were collected into vials, containing 2.5 % potassium dichromate, and before examination stored at room temperature (23°C) for five days to sporulate. Faecal sedimentation was performed using a modified technique described by FOREYT (2001). Eggs and oocysts of parasites were identified microscopically based on their size and morphologic characteristics.

Results and discussion

The total occurrence of intestinal parasitic infection among 350 animals in Zoo Ljubljana was 42.6 % (table 1); with 26.6 % for helminthes, 7.7 % for protozoa and 8.3 % for mixed infection of both helminthes and protozoa. The group of helminthes was mostly represented with gastrointestinal strongyles, *Capillaria* spp. and *Trichuris* spp. The group of protozoa was represented with *Balantidium coli*, *Eimeria* spp. and *Isospora* spp. Table 2 shows how many species of one group of animals was infected in particular year. According to this data we can summarise that parasites infections and infected hosts vary between groups and years.

This study reported that among infected primates, there was a higher occurrence of helminthes (15 %) compared to protozoa (5 %). The different results were obtained in Zoo Negara in Malaysia by LIM *et al.* (2008) with higher prevalence of protozoa (35.4 %) compared to helminthes (19.1 %); similarly with higher prevalence of protozoa than helminthes in hoofed mammals. Among all tested groups of animals, they detected the highest parasitic infection (89.3 %) in felines (LIM *et al.*, 2008). In Zoo Ljubljana, parasite infection was recorded in carnivorous during years 1980 and 1995 (EMERŠIČ, 1996), however this group of animals were negative for whole period in our present study. This fact could be due to limited transport of animals during last two decades and precise quarantine protocol.

Tab. 1: Total occurrence (%) of intestinal parasitic infection among animals of different groups in Zoo Ljubljana.

Animal groups	Number of samples	Helminthes positive (%)	Protozoa positive (%)	Helminthes and protozoa positive (%)	Total
Hoofed mammals	183	58 (31.7 %)	19 (10.4 %)	23 (12.6 %)	100 (54.6 %)
Domestic animals	33	8 (24.2 %)	6 (18.2 %)	3 (9.1 %)	17 (51.5 %)
Autochthonous	78	28 (35.9 %)	7 (9.0 %)	17 (60.7 %)	52 (66.7 %)
Allochthonous	72	22 (30.6 %)	6 (8.3 %)	3 (4.2 %)	31 (43.1 %)
Reptiles	82	22 (26.8 %)	5 (6.1 %)	6 (7.3 %)	33 (40.2 %)
Primates	40	6 (15 %)	2 (5.0 %)	0 (0 %)	8 (20 %)
Ratites	25	7 (28 %)	1 (4.0 %)	0 (0 %)	8 (32 %)
Carnivores	20	0 (0 %)	0 (0.0 %)	0 (0 %)	0 (0 %)
Total	350	93 (26.6 %)	27 (7.7 %)	29 (8.3 %)	149 (42.6 %)

Tab. 2: Total occurrence (%) of intestinal parasitic infection among species of different groups according to different years of sampling.

Animal groups	N	2008	2009	2010	2011	2012
Hoofed mammals	16	3 (18.8 %)	6 (56 %)	9 (56 %)	11 (68.8 %)	14 (88 %)
Domestic animals	5	1 (20 %)	1 (20 %)	2 (40 %)	2 (40 %)	4 (80 %)
Autochthonous	5	1 (20 %)	3 (60 %)	5 (100 %)	5 (100 %)	5 (100 %)
Allochthonous	6	1 (17 %)	2 (33.3 %)	2 (33.3 %)	4 (66.7 %)	5 (83.3 %)
Reptiles	10	5 (50 %)	3 (30 %)	6 (60 %)	2 (20 %)	3 (30 %)
Primates	5	0 (0 %)	2 (40 %)	2 (40 %)	1 (20 %)	1 (20 %)
Ratites	3	0 (0 %)	1 (33.3 %)	0 (0 %)	1 (33.3 %)	2 (66.6 %)
Carnivores	5	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)
Total	39	8 (20.5 %)	12 (30.8 %)	17 (43.6 %)	15 (38.5 %)	20 (51.8 %)

N – number of species in a group

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HUMAN METAPNEUMOVIRUS OUTBREAK IN A GROUP OF CHIMPANZEES (*PAN TROGLODYTES*) IN ZOO LJUBLJANA

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Human metapneumovirus (HMPV) primary causes an acute respiratory tract infection in human. Chimpanzees (*Pan troglodytes*) are sensitive for many viral pathogens of human origin. A single group of six captive chimpanzees in Zoological Garden Ljubljana started to show clinical signs of upper respiratory tract disease which rapidly progressed into difficult breathing with higher respiratory rate and coughing in younger animals. Even though antibiotic therapy was started promptly after severe tachypnoea occurred, the youngest animal died 12 hours later. Immediate autopsy and laboratory testing revealed severe bronchopneumonia caused by human metapneumovirus and secondary bacterial infection with *Klebsiella pneumoniae*. Second and last fatality occurred on day three of 14 days long outbreak, when 11 year old castrated male died during the anaesthesia induction. Pathological findings were identical and metapneumovirus was confirmed as viral etiological agent. All remaining group members were positively tested for metapneumovirus and they showed clinical improvement after one week of treatment. Complete resolution of clinical signs was obvious by day 14. According to enclosure set up, viral droplet contact from visitors is to be suspected as a way of transmission. Thorough examinations of all factors prior and during the outbreak are important for prevention of this zoonosis in the future.

GASTROINTESTINAL AND RESPIRATORY PARASITES SURVEY IN WILD AFRICAN LIONS (*PANTHERA LEO*) FROM NIASSA NATIONAL RESERVE, MOZAMBIQUE – PRELIMINARY RESULTS

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Summary

The African lion (*Panthera leo*) is an iconic species of the African continent, classified as vulnerable by the IUCN. Infectious diseases are one main threat for the survival of this species. However, the impact of parasitic infections has been overlooked and poorly documented. In order to characterise the parasitological fauna of these animals, a study was carried out in the Niassa National Reserve (NNR), Northern Mozambique, where a large population of lions lives. In partnership with the Niassa Lion Project and the administration of the Niassa Reserve, 44 lion faecal samples were collected in an area of 600 km² (Concession L5-South), and later processed and analysed at the Laboratory of Parasitology and Parasitic Diseases, Faculty of Veterinary Medicine, Lisbon University. Results show that 65.9 % (29/44) of the samples were infected with parasites, namely 47.7 % for *Toxocara* sp., 31.8 % for *Aelurostrongylus* sp., 25 % for *Spirometra* sp., 27.3 % for Taeniidae, 18.2 % for *Paramphistomum* sp. and 13.6 % for *Linguatula* sp. Out of the 29 positive samples, 72 % (21/29) were co-infected, registering double infection in 21 % (6/29), triple in 34 % (10/29), quadruple in 10 % (3/29) and fivefold in 7 % (2/29). These results are consistent with previous studies performed in the African continent, with the exception of *Linguatula* sp., which had not yet been reported in wild lions.

Introduction

The Niassa National Reserve (NNR) is one of the largest, most remote and least known conservation areas in the World, located in the far North of Mozambique. This Reserve stretches over 42,000 km², representing the conservation area with the highest density of wildlife in the country, with particular relevance for its African lion population (*Panthera leo*), with an estimated size of 1000 to 1200 individuals (NIASSA CARNIVORE PROJECT, 2013). For this reason, the Niassa National Reserve is considered a stronghold for the species (RIGGIO *et al.*, 2012) and represents one of the few areas in the World where the species has a chance of long-term survival. According to The IUCN Red List of Threatened Species, *P. leo* is mostly in decline throughout its range. The African lion is a species classified as vulnerable by the IUCN and is protected by CITES Appendix II. The main threats to its survival are conversion and destruction of habitat, prey depletion and indiscriminate killing (IUCN, 2014). Diseases may contribute to the reduction of animal populations and biodiversity loss. There is currently little information on parasitic diseases in wild lions, although their parasitological fauna is considered to be very different from that reported in lions kept in zoos (SCOTT, 1988; MÜLLER-GRAF, 1995). In order to characterise the diversity and the level of parasitism of African lions in NNR, a study

was developed in a partnership with the Niassa National Reserve Management authority (a co-management agreement between Wildlife Conservation Society and the Ministry of Tourism) and the Niassa Lion Project, which has been based in Niassa National Reserve since 2003.

Material and methods

Between October and November 2014, 44 lion faecal samples were identified and collected from the field, in an area with approximately 600 km² (L5-South Concession) of the NNR. Multiple sampling of the same animal(s) was possible, as the source of faecal samples was not known. Samples were located by a local tracker and lion field worker, stored in dry conditions, transported in plastic bags to the base camp of Niassa Lion Project and kept in a cool place until transported by plane to Portugal. Samples were analysed between 15 to 30 days after collection at the Laboratory of Parasitology and Parasitic Diseases, CIISA-FMV-ULisboa, using the following coprological methods according to THIENPONT *et al.* (1986) and BOWMAN (2014): qualitative natural sedimentation and Willis flotation (for isolating helminth eggs and protozoan oocysts); McMaster quantitative method (to assess the number of eggs per gram of faeces, EPG); and Baermann technique (for the search of first stage larvae (L1) of pulmonary nematodes).

Results

A total of 65.9 % (29/44) of the samples were positive considering all the coprological techniques. A wide range of parasites was observed (figure 1), belonging to genus *Toxocara* 47.7 % (21/44), *Aelurostrongylus* sp. 31.8 % (14/44), *Spirometra* sp. 25 % (11/44), *Taeniidae* family 27.3 % (12/44), *Paramphistomum* sp. 18.2 % (8/44) and *Linguatula* sp. 13.6 % (6/44).

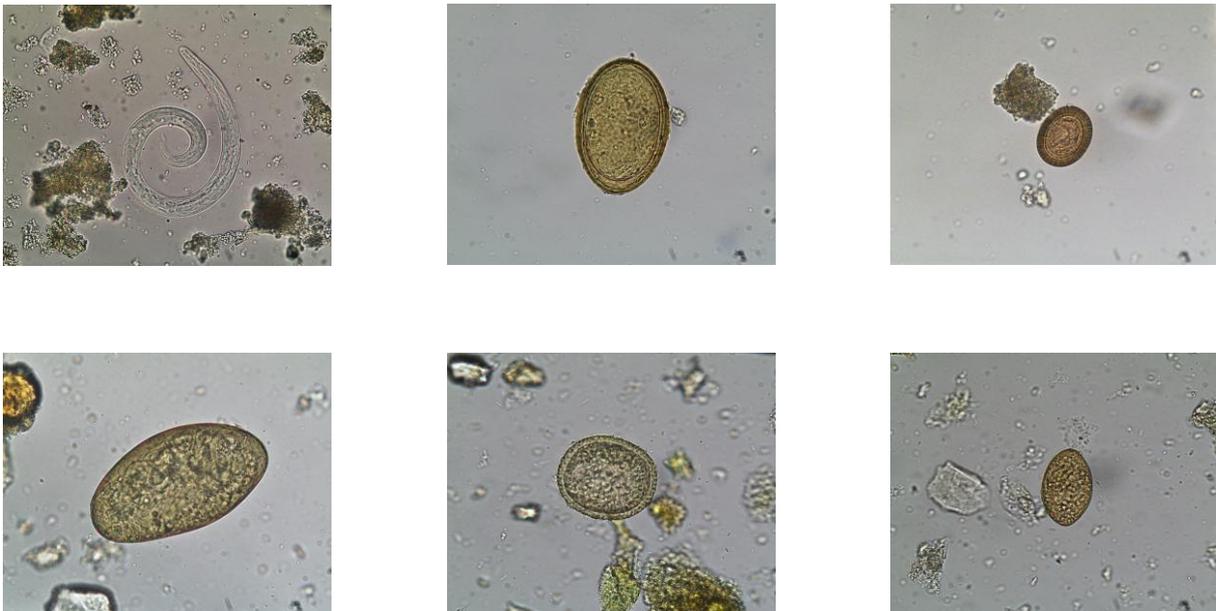


Fig. 1: Left to right, top row: *Aelurostrongylus* sp. (L1 Larvae - 260 µm x 2.5 µm, 40x), *Linguatula* sp. (Egg - 80 µm x 53 µm, 40x), *Taeniidae* (Egg - 39 µm x 32.5 µm, 40x). Bottom row: *Paramphistomum* sp. (Egg - 120 µm x 67.5 µm, 40x), *Toxocara* sp. (Egg - 63 µm x 55 µm, 40x), *Spirometra* sp. (Egg - 57 µm x 35 µm, 40x). (Photos: Lajas LM)

Out of the 29 positive samples, 28 % (8/29) were infected by a single species of parasite and the remaining 72 % (21/29), were co-infected with two, 21 % (6/29), three, 34 % (10/29), four, 10 % (3/29) and even five, 7 % (2/29) distinct parasites. The most common co-infection was the one caused by *Aelurostrongylus* sp. and *Toxocara* sp., 57 % (12/21).

Discussion

The number of parasite species identified in this study was higher than previously found in Namibia by SMITH and KOK (2006) and lower than those found in Zambia by BERENTSEN *et al.* (2012) and in Tanzania by MÜLLER-GRAF (1995) and BJORK *et al.* (2000). *Toxocara* sp. (47.7 %) was the most prevalent parasite in our study, in comparison with the studies of MÜLLER-GRAF (1995), MÜLLER-GRAF *et al.* (1999) BJORK *et al.* (2000) and BERENTSEN *et al.* (2012), who documented *Spirometra* sp. as the most common parasite. The registered prevalence of 32 % for *Aelurostrongylus* sp. in this work was higher than previously described 20 % (BJORK *et al.* 2000) and 21 % (BERENTSEN *et al.*, 2012). The prevalence of Taeniidae eggs, 27.2 %, is intermediate between BERENTSEN *et al.* (2012) (80 %), MÜLLER-GRAF *et al.* (1999) (58 %) and BJORK *et al.* (2000) (15 %). The prevalence of 13.6 % for *Linguatula* sp. represents the first documentation of this parasite species in *P. leo* in the wild, since only two authors reported this parasite as a potential cause of disease in African lions (YOUNG, 1975; MUKARATI *et al.*, 2013). The presence of *Paramphistomum* sp., was considered pseudo-parasitism, as a result of hunting wild ruminants, in particular buffalos. The diet of these big cats is probably the source of most of the parasitic forms found, although it is not possible to establish a direct link between the identified parasites and the prey species they belong to. *Toxocara* sp. was the most prevalent parasite, probably due to its direct life cycle, but also due to the chance of ingesting paratenic hosts with larval stages. The parasite species found in this study may have been influenced by the weather condition of the dry season (with extremely high temperatures and very low humidity), causing dehydration of the samples taken from the field. This fact probably mirrors a potential sub-characterisation of parasitic load, suggesting that prevalence of some parasites found in this study may be higher. Ancylostomatid eggs with its thin capsule were not found, probably because high temperatures made impossible the survival of hookworm eggs. However, the high prevalence of *Toxocara* sp. (a parasite with direct life cycle) and *Aelurostrongylus* sp. (with indirect life cycle) is remarkable. That could be related also with high animal density in that area, or probable inter-transmissibility of these nematodes among big carnivores living in this ecosystem. Wild animals can be natural hosts of many parasite species and other infectious agents, which usually do not cause disease until other triggering factors arise (MUNSON *et al.*, 2008). Due to their life cycles and host-parasite interactions, we consider high loads of *Toxocara* sp. and *Aelurostrongylus* sp. to be a potential cause of disease in lions. Further studies on the pathological effects of these parasites in wild lions are needed. The identification of the parasitic elements was based on morphometric analysis and comparison with current literature, which allowed the identification until genus level. Molecular analysis is under progress in order to enable an accurate and complete identification. This qualitative study represents the first survey on the population of African lions from NNR and Mozambique and is also one of the few ever undertaken within the parasitology of African lions in the wild.

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AIR SAC PAPILLARY CARCINOMA IN A BAR-HEADED GOOSE (*ANSER INDICUS*)

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A 21 year old captive-born male bar-headed goose (*Anser indicus*) was found dead without preliminary symptoms. A full necropsy was performed and revealed marked emaciation and the presence of a 10 x 8 x 5 cm mass in the cranial coelomic cavity; this mass was firm, infiltrating the keel, displacing caudally all internal organs, and compressing the lungs and heart. Histopathology of this mass showed a papillary epithelial proliferation composed of numerous irregular ramifications of conjunctivovascular stroma; cells had a moderate eosinophilic cytoplasm. Anisocytosis and anisocaryosis were mild. Lesions were consistent with an air sac papillary carcinoma. The other lesions revealed by histopathology were marked epithelial hyperplasia of air sacs, marked multicentric congestion, and marked pulmonary congestion, oedema and atelectasis.

Papillary carcinomas of air sac origin are locally invasive and may present as external masses. Classically, they primarily develop in humeral air sacs. This neoplastic process is scarcely reported, and previous cases have been described in cage birds (MARSHALL, 2004; REAVILL, 2004). In a retrospective study realised between 1994 and 2002, on the 557 tumours diagnosed in birds, 9 (1.6 %) were air sac carcinomas. All the cases reported showed an invasive behaviour, metastases were found in 4/9 (44.4 %) cases, and sac carcinomas were responsible for the death of 8/9 birds (88.9 %) (HARRISON and LIGHTFOOT, 2006). Order Anseriformes had a prevalence of 11.6 % for tumour processes (119/1024 cases), considerably higher than the average for tumour submissions from other bird orders. All the birds were adults or aged and most tumours likely occurred spontaneously (HARRISON and LIGHTFOOT, 2006). To the authors' knowledge, this is the first case of air sac carcinoma in a bar-headed goose.

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COLONIC ADENOCARCINOMAS IN A GROUP OF CAPTIVE AMUR RAT SNAKES (*ELAPHE SCHRENCKI*)

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Nine cases of colonic adenocarcinomas in a familial group of captive Amur rat snake (*Elaphe schrencki*) were diagnosed at the Réserve Africaine de Sigean, France, between 1986 and 2013. The tumour type was confirmed by histopathology in five cases. Colonic neoplasms were detected in seven females and two males, all adults, and accounted for 29 % of causes of death in adults of this species at this institution. Clinical signs ranged from spontaneous death without preliminary symptoms, anorexia, coprostasis or egg binding, to marked abdominal distension or cloacal mass. Grossly, mild to marked thickening of the intestinal wall cranially to the cloaca was found in seven cases; tan to yellow firm masses were noted in the distal intestinal wall in the other two cases. Microscopically, neoplasms were characterised by infiltrating, poorly circumscribed and unencapsulated nests of epithelial cells. Marked anisokaryosis and anisocytosis were seen in all investigated neoplasms. Moderate polymorphic inflammation was present in four cases. No metastases were detected macroscopically or microscopically in all cases examined.

Though neoplasia is commonly encountered in reptiles, spontaneous tumours of the gastrointestinal tract are uncommon. Intestinal adenocarcinomas have been previously described in captive and free-ranging snakes including rat snake (*Elaphe obsoleta*) (RAMSAY and FOWLER, 1992), bull snake (*Pituophis sayi*) (COWAN, 1968), corn snake (*Elaphe guttata guttata*) (LATIMER and RICH, 1998), Burmese python (*Python molurus bivittatus*) (CHANDRA *et al.*, 2001), gopher snake (*Pituophis melanoleucus*) (JESSUP, 1980), and emerald tree boa (*Corallus caninus*) (OROS *et al.*, 2004). To the authors' knowledge, this is the first report of colonic adenocarcinoma in Amur rat snakes. The prevalence of this tumour in this familial group emphasises possible familial and hereditary factors, as identified in man. Inbreeding in snakes should be avoided to prevent increased tumour incidence.

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PATENT DUCTUS ARTERIOSUS IN A GREATER KUDU (*TRAGELAPHUS STREPSICEROS*)

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A 3.5 month old captive-born male greater kudu (*Tragelaphus strepsiceros*) was presented for sudden apathy. Physical examination revealed good body condition, marked apathy, mild dehydration, and a continuous machine-like heart murmur of grade 4/6. Clinical signs were consistent with shock, potentially with acute heart failure, and this animal received supportive symptomatic treatment. It was found dead on the next day. A necropsy was performed and showed a 2 mm in diameter communicating blood vessel between the aorta and the pulmonary artery, 4 cm cranial to the base of the heart, consistent with a patent ductus arteriosus. The other main macroscopic lesions were multicentric congestion and necrosis of the ear tips. The main lesions revealed by histopathology were mild congestion of all internal organs, marked pulmonary oedema, and a severe necrosuppurative auricular dermatitis. It is important to note that there was no histological evidence of significant cardiac dysfunction, suggesting no major impact from the patent ductus arteriosus, and that the latter was not the cause of death. The ductus arteriosus is a normal foetal blood vessel that should close soon after birth. It can be found alone or in combination with other cardiac defects such as interatrial or interventricular connections, or tetralogy of Fallot (MAXIE and ROBINSON, 2007). Patent ductus arteriosus is frequently reported in domestic ruminants (CEBRA and CEBRA, 2002; MAXIE and ROBINSON, 2007). In zoo medicine, this condition has previously been reported: a Sumatran tiger (*Panthera tigris sumatrae*) (BARNES *et al.*, 2001), lions (*Panthera leo*) (PIZZI *et al.*, 2007), and an adult Amur leopard (*Panthera pardus orientalis*) (DOUAY *et al.*, 2013), but are scarce in zoo ruminants. Extrapolated from cattle, this condition could be due to a viral infection or metabolic dysfunction of the dam, foetal anoxia, some toxins or drugs, nutritional deficiencies or of genetic origin (CEBRA and CEBRA, 2002; MAXIE and ROBINSON, 2007); this last cause is suspected in our case as this animal's coefficient of inbreeding is more than 12.5 %. To the authors' knowledge, this is the first reported case of patent ductus arteriosus in a greater kudu.

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IMMOBILISATION OF CAPTIVE NUBIAN IBEX (*CAPRA NUBIANA*) WITH BUTORPHANOL-MIDAZOLAM-MEDETOMIDINE OR BUTORPHANOL-AZAPERONE-MEDETOMIDINE AND ATIPAMEZOLE REVERSAL

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Seventeen captive Nubian ibex (*Capra nubiana*) were immobilised for transportation and/or hoof trimming, deworming, and vaccinations. Of these, 11 were immobilised with a combination of butorphanol (0.13 ± 0.03 mg/kg) (Torbugesic, Fort Dodge Laboratories, Fort Dodge, IA, USA), midazolam (0.13 ± 0.03 mg/kg) (Midolam 15, Rafa Laboratories, Jerusalem, Israel), and medetomidine (0.13 ± 0.03 mg/kg) (Medetomidine, Kyron Laboratories, Johannesburg, South Africa) (BMM), and six were immobilised with a combination of butorphanol (0.11 ± 0.03 mg/kg), azaperone (0.22 ± 0.06 mg/kg) (Azaperon, Kyron Laboratories, Johannesburg, South Africa), and medetomidine (0.11 ± 0.03 mg/kg) (BAM) by intramuscular injection. Induction and recovery times were recorded. Heart rate, respiratory rate, rectal temperature, blood pressure, and oxygen saturation were measured. The quality of induction, immobilisation, and recovery were scored (scale 1 - 5; 1 = poor, 5 = excellent). Mean induction time was significantly shorter in the BMM group versus the BAM group (8.8 ± 2.7 and 20.1 ± 7.8 min, respectively). Median induction score and median immobilisation score were significantly higher (i.e., better) in the BMM group versus the BAM group (5 versus 2.5, and 4 versus 3, respectively). The mean and diastolic blood pressures were significantly higher in the BMM group at the 25 min time point. Atipamezole (Antisedan[®], Pfizer Animal Health, Exton, PA, USA) was administered at the end of procedures, and all ibex recovered smoothly. Mean recovery time was significantly longer in the BMM group versus the BAM group (9.5 ± 4.3 and 3.3 ± 2.2 , respectively). In conclusion, at the doses used, the combination of BMM was superior to BAM for short term immobilisation in captive Nubian ibex.

ANTIMICROBIAL SUSCEPTIBILITY OF *ESCHERICHIA COLI* STRAINS ISOLATED FROM *ALOUATTA* SPP. FAECES TO ESSENTIAL OILS

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Essential oils are secondary metabolites of plants with different biological properties, such as activity against microorganisms. Nowadays, essential oils have been used in animal feed to combat infection and improve animal productivity. This study aimed to evaluate the *in vitro* antibacterial activity of essential oils from *Lippia graveolens* (Mexican oregano), *Origanum vulgare* (oregano), *Thymus vulgaris* (thyme), *Rosmarinus officinalis* (rosemary), *Cymbopogon winterianus* (citronella), *Cymbopogon citratus* (lemon grass) and *Eucalyptus globulus Labill* (eucalyptus) against *Escherichia coli* ($n = 22$) strains isolated from captive *Alouatta* spp. faeces. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined for each isolate by broth microdilution technique, from the maximum concentration of 6400 µg/ml. Mexican oregano oil showed a better antibacterial activity (MIC mean = 1818 µg/ml; MBC mean = 2618 µg/ml), compared to thyme oil (MIC mean = 2618 µg/ml, MBC mean = 2909 µg/ml) and oregano oil (MIC mean = 3418 µg/ml, MBC mean = 4800 µg/ml). The essential oils of eucalyptus, rosemary, citronella and lemon grass did not show any antibacterial activity. Other studies also reported the antibacterial activity of the Mexican oregano, thyme and oregano oils against *E. coli*. These results confirm the potential of the antimicrobial effect of some essential oils, which deserve further research.

UTERINE ADENOCARCINOMA IN AN ASIAN ELEPHANT (*ELEPHAS MAXIMUS*)

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A 56 year old nulliparous female Asian elephant (*Elephas maximus*) was found in lateral recumbency (agonal state) due to a cardiovascular collapse and humane euthanasia was elected. *Post mortem* examination was performed in order to assess the cause of death. Macroscopically, the uterine body was moderately increased in size and firmer than normal. The lumen was reduced due to a poorly demarcated and infiltrative neoplasm showing papillary and soft luminal projections and multiple brownish necrohaemorrhagic areas. Furthermore, multiple, disseminated, whitish to grey, well demarcated neoplastic nodules, ranging from 2 - 5 cm in diameter, involving both uterine horns and the uterine body were observed. Multiple whitish firm nodules, 1 - 4 cm in size were present in both lungs. Bulging into the right cardiac ventricle, and adhering to the endocardium, a firm to elastic reddish thrombus 3 cm in diameter was present. Based on the macroscopic examination, a uterine adenocarcinoma with endocardial and pulmonary metastasis and multiple uterine leiomyomas was suspected. Representative samples of all organs were collected for a full histopathologic examination and immunohistochemical analysis. Microscopically, an infiltrative unencapsulated and variably cellular neoplastic proliferation composed of epithelial cells and separated by abundant well vascularised myxoid to fibrous stroma, had infiltrated the endometrium and the external muscular layer. The neoplastic cells formed acinar and tubular structures, solid aggregates and were polygonal with distinct cell borders and eosinophilic to light basophilic cytoplasm. The nuclei were oval with marginated or granular chromatin with a single prominent nucleolus. Cellular atypia consisted of marked anisokaryosis and anisocytosis. Mitotic figures ranged from 2 – 4/400x magnification field. Multifocal aggregates of neoplastic cells were noted in myometrial lymphatic vessels. Neoplastic cells showed a strong cytoplasmatic immunoreactivity to pancytokeratin antibody. Large areas of coagulative necrosis, haemorrhages and peripheral lympho-plasmacytic inflammation were observed. Multiple nodular uterine masses consisted of a relatively uniform population of mesenchymal cells arranged in interlacing bundles or whorls and supported by a moderate amount of fibrovascular stroma. The neoplastic cells were spindle-shaped, often showed cigar-shaped nuclei and a mild to moderate anisokaryosis and anisocytosis; mitotic figures were rare. Neoplastic cells were strongly positive to smooth muscle actin antibody. Pulmonary lesions recapitulated the histological and immunohistochemical (IHC) features of the epithelial uterine neoplasm. Aggregates of neoplastic cells were found in mediastinal lymph nodes. The histological and IHC feature of the intraventricular mass revealed a markedly atypical population of signet-ring, pancytokeratin positive, epithelial cells, occasionally arranged in acinar structures and mixed within a large amount of polymerised fibrin, erythrocytes, few granulocytes and lymphocytes. Collectively, histological data confirmed the macroscopic suspect diagnoses. The final diagnoses were uterine adenocarcinoma with intraventricular and pulmonary metastases and multiple uterine leiomyomas. Uterine leiomyoma is the most common uterine neoplasm in domesticated Asian elephant; on the contrary, to the author knowledge, this is the first report of an uterine adenocarcinoma with distant metastases in an Asian elephant. Based on *post mortem* results have been supposed that the recurring colics were due to the enlarged uterus and compression on the intestine and that one of this colic caused the recumbency and cardiovascular collapse.

EFFECTS OF MORPHINE ON THE SPONTANEOUS LOCOMOTOR ACTIVITY IN TEGUS (*SALVATOR MERIANAE*)

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The aim of this study was to evaluate the influence of morphine on the spontaneous locomotor activity (SLA) in tegus (*Salvator merianae*). Seven tegus weighing 652 ± 272 g, maintained in a controlled environment (12/12; 29°C; relative humidity 50 %), were submitted to three intramuscular treatments: M5 – 5 mg/kg of morphine; M10 – 10 mg/kg of morphine, and Control - 0.5 ml of NaCl 0.9 %. The SLA was assessed with an open field test, and was recorded for 15 min by a video camera, at 0 min (baseline), 30 min, 1, 2, 3, 4, 6, 12, and 24 h post-treatment. The images were analysed in a random order by two observers blinded to the treatments. Body core temperature was also measured at the same time points. Statistical analysis were done by ANOVA followed the Bonferroni's test (group to baseline), and two-way ANOVA followed the Tukey's test (among groups, each moment), $P < 0.05$. The results showed a reduction in body core temperature in all groups, only at 12 h post-treatment. There was no difference in the SLA in control group along the moments, except at 12 h post-treatment. The tegus showed low SLA at 30 min to 4 h and at 12 h in M5, and 30 min to 24 h in M10. Comparison between groups showed that morphine reduces SLA in a dose-dependent manner, up to 6 h after treatment. Therefore, a single-dose of morphine administration reduces SLA in a dose-dependent manner.

DIAGNOSIS, TREATMENT AND CLINICAL SIGNS OF *SAPROLEGNIA* SP. INFECTION IN OLMS (*PROTEUS ANGUINUS*)

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Summary

Proteus anguinus (the olm) is the only real cave dwelling vertebrate found in Europe, and an amphibian endemic to subterranean caves of the Dinaric carst.

Despite of its popularity, a general knowledge of the olm's ecology and biology is limited. Because of that reason, Croatian Herpetological Society together with its partners started the research and conservation project on the olms, which includes the field and *ex situ* research. For the purpose of *ex situ* research, flushed out animals and animals from their natural habitats are kept in specially designed cold room situated at the back stage of Zagreb Zoo. Two years ago, fluffy changes appeared over the whole body of nine flushed out animals and despite the treatment introduced immediately, they died within five days after symptom appearance. The standard microbiology and histology of the skin of the dead animals revealed the presence of *Saprolegnia* sp. infection in all animals. Other pathogens such as Ranavirus, *Batrachochytrium dendrobatidis* and *Chlamydia* sp. were ruled out both by molecular methods and histology.

Introduction

Proteus anguinus is the only species in the *Proteus* genus and only European species of the *Proteidae* family. This very special amphibian is adapted to a life of complete darkness in their underground habitats in Dinaric caves (DUMAS and CHRIS, 1998). The olms are endemic to the water biotopes of Dinaric karst inhabiting areas from northern Italy, southern Slovenia and south-western Croatia and Bosnia and Herzegovina (SKET, 1997). The olms are slender and gentle amphibians. Their average body length is 23 - 25 cm. Their eyes are poorly developed and completely covered by the skin. Although their skin is translucent they do not display albinism, since the animals can gain black area on the body when exposed to light. Due to their specific way of life, they are highly dependent on clean water and therefore very susceptible to the human impact on their habitats (BULOG *et al.*, 2001).

To gain as much as possible information about the biology, behaviour and microbiology of the olms, Croatian Herpetological Society in association with its partners initiated the project „*Proteus anguinus* in Croatia – conservation research project“ three years ago. The project is composed of the field research and conservation and of *ex situ* research based on keeping the animals in specially designed „cold room“ which ensures all the needs of the animals regarding the temperatures of the air and water, food, aeration and shelters. The majority of animals kept in the “cold room” are those flushed out from their natural habitats at certain localities in Croatia due to heavy rains or snow melting.

Because of unnatural conditions on the fields, these animals are immunocompromised and more prone to various types of opportunistic pathogens. Some of the kept animals were taken from their natural habitats for scientific purposes, to compare the behaviour and microbiological findings with the animals being flushed out.

Here we present clinical signs, therapy, and diagnostic approaches to animals infected with *Saprolegnia* sp.

Cases

Nine flushed out animals were captured, brought to the Zagreb Zoo and immediately clinically examined. Oral cavity, cloacal and skin swabs were taken for standard microbiology. Swabs from oral cavity and cloaca were taken also for the detection of *Chlamydia* sp. by the real time PCR method, and skin swabs were taken for detection of *B. dendrobatidis* and Ranavirus. After completion of veterinary procedures, all of the animals were situated individually in glass aquariums. Water from aquariums was previously tested by standard microbiology and water conductivity. Two weeks after their arrival, five animals were presented with white fuzzy changes over the whole skin surface including the gills, within 24 h. General condition of the animals was bad, meaning that some of the animals were not moving at the bottom of aquaria, and some were gasping for the air on the surface. Skin swabs were taken, and the treatment with Itrakonazole 0.02 % (Itrafungol 10 mg/ml, Elanco, Bad Homburg, Deutschland) in 0.6 % Ringer solution (prepared by CRAWSHAW, 1998) started immediately in the form of 15 min daily baths. Despite of the therapy all of the animals died within five days. Two flushed out animals from the other locality gained the same symptoms after one year of captivity, although no changes in their husbandry or similar stressors were noticed, and also died. Necropsy was performed on all dead animals, and the organs were taken for histological examination.

Results

Saprolegnia sp. was confirmed in all dead animals both with standard microbiological procedures of skin swabs and histologically from the skin of all dead animals. Skin swabs of all animals were negative for *B. dendrobatidis* and Ranavirus, while oral cavity and cloacal swabs were negative for *Chlamydia* sp. Low number of soil bacteria such as *Pseudomonas fluorescens*, *Escherichia coli* and *Bacillus* sp. was isolated from the skin, oral cavity and cloaca of all animals. In three out of nine dead animals *Aeromonas hydrophila* was isolated from oral cavity, cloaca and skin swabs. The water from aquariums shows only a low number of bacteria such as *Micrococcus* sp. and *Bacillus* sp. Regarding the histological findings, occlusion of the kidney tubules by myxozoan plasmodia and/or degeneration of the epithelial cell layers were found in three animals.

Discussion

Till now, 23 animals were kept or still live at our facility for research. Among them 16 were flushed out animals and 7 were taken from their natural habitats at different parts of Croatia. All of the animals are kept under the same conditions and different kinds of microbiological tests are performed to collect as much as possible information about their pathogens as well as their normal and opportunistic microflora. We never detected the presence of *B. dendrobatidis*, Ranavirus or *Chlamydia* sp. in any animals. In the standard bacteriology the most common isolates were soil bacteria such as

Pseudomonas sp., *Bacillus* sp. and *Aeromonas hydrophila*. Although *Aeromonas hydrophila* could be one of potential cause of red leg disease in amphibians (SCHADICH and COLE, 2010), we never noticed this problem in the olms. The life cycle and the route of infection of myxozoa found in the olms is still unknown, but to our knowledge, there is no plausible interrelation between this parasites and the occurrence of *Saprolegnia*.

Since this kind of symptoms occurred only in the flushed out animals, we suppose that the unnatural conditions on the field together with the *Saprolegnia* sp. from freshwater and organic material are responsible for the disease (ROMANSIC *et al.*, 2009; TERREL *et al.*, 2013).

None of the animals from their natural habitats gained the similar symptoms, indicating that the flushed animals are more prone to opportunistic microorganisms, especially when exposed to unnatural conditions on the field. To our knowledge, this is the first report on *Saprolegnia* sp. infection in the olms. Much more information on diagnosis, treatment and conditions to keep the animals in good health is needed.

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SCREENING OF SELECTED PATHOGENS AND GENERAL HEALTH STATUS OF FRESHWATER TURTLES (*TRACHEMYS* SP.) INTRODUCED TO CROATIA

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Pathogens introduced by invasive species can be the cause of decline of native species, but may also be a source of many zoonoses. As in the other parts of the world, *Trachemys* species illegally released to nature by their owners represent a great problem in Croatia. There is a lack of information about pathogens related to the released *Trachemys* sp. in Croatia, but also in Europe. Therefore we monitored released animals from a pond situated near the capital city centre for selected pathogens.

A total of 30 turtles from the same pond were captured manually with hand nets, brought to the Faculty of Veterinary Medicine and housed for two days to allow the sampling. Physical examination of each turtle was performed, including weighing, measurement of the carapace length, inspection of visible mucous membranes, shell and the skin, palpation of the coelom through the prefemoral fossa, and sex determination. Sterile swabs were taken from the oral cavity and cloaca of each animal for standard microbiology. Cloacal swabs were additionally enriched with Selenite broth for *Salmonella* sp. detection. Cloacal swabs were also used for *Campylobacter* sp. detection using the ISO method. Pooled oral cavity and cloacal swabs of each animal were used for *Chlamydia* sp. and Ranavirus detection by real-Time PCR methods. Blood samples taken from the dorsal tail vein were tested for the presence of specific antibodies against 12 *Leptospira* serovars using a microscopic agglutination test (MAT). In addition, haematology and detection of blood parasites of each animal were performed. According to their secondary sex characteristics, 18 animals were females and 12 were males. Regarding the carapace length all of them were adults. Some of them were in bad condition, as judged by a weak musculature, sunken eyes, and the colour of the oral cavity mucous membranes. The haematologic evaluation included packed cell volume (PCV), total erythrocyte count (RBC), differential leukocyte count and morphological erythrocyte and lymphocyte assessment. In 15 animals PCV was less than 15 %, RBC was lower than $0.3 \times 10^6/\mu\text{l}$ in 26 animals. In the majority, the most common leukocytes were heterophils and lymphocytes while in three animals the dominate cells were basophils. In two animals polychromasia and immature erythrocytes were found. Blood parasite examination was performed on Giemsa stained thin blood films. No blood parasites were found.

All of the animals were *Salmonella* sp. and *Campylobacter* sp. negative, although different opportunistic gram negative bacteria were isolated both from the oral cavity and cloaca. All animals were also negative for Ranavirus, but 20 out of 30 animals were positive for *Chlamydia* sp. Antibodies against four pathogenic *Leptospira* serovars were detected in 5 of 30 turtles at titres ranging from 1:100 to 1:400.

This pilot study indicates that invasive imported species could have the potential to impact the health of other animal species or eventually humans, so that the screening is advisable. To confirm this, larger scale investigation on more locations, involving more animal species will be necessary.

SEROPREVALENCE OF *TOXOPLASMA GONDII* IN ZOO AND CIRCUS ANIMALS IN ITALY

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Summary

Animals from zoos and circuses could be very sensitive to *Toxoplasma gondii* infection which can lead to abortion or even to death. The aim of present study was to determine the prevalence of antibodies to *T. gondii* in 69 mammals of six families collected in three zoos and four circuses in Southern Italy. The animals were divided into groups according to different criteria (family, gender, age, country of origin, place where they are bred, kind of housing and contact with cats). Antibodies to *T. gondii* were found by indirect fluorescence antibody test in 34 (49.3 %) of both zoo and circus animals with titres 50 - 3200. The high seroprevalence 95.5 % was found in Felidae (21/22), 30.4 % in Camelidae (7/23), 20 % in Bovidae (2/10), while Equidae were negative (0/8). *T. gondii* antibodies were also detected in Cervidae (2/2) and Hominidae (2/4). Animals from circuses (58.1 %) were considered more positive than animals from zoos (34.6 %). Since *T. gondii* prevalence in zoo and circus animals in Southern Italy is very high (49.3 %), it is recommended to continue with prevalence studies as well as to keep animals in good hygienical conditions.

Introduction

The clinical manifestation of *T. gondii* infection was noticed in many zoos all over the world. Lethal form of toxoplasmosis was recorded e.g. in *Octocolobus manul* (RIEMANN *et al.*, 1974), Nilgais (*Boselaphus tragocamelus*) or Saiga antelopes (*Saiga tatarica*) (SEDLÁK *et al.*, 2004). Clinical form of toxoplasmosis was recorded also in primates *Lemur catta* or *Saimiri sciureus* that suffered for diarrhoea, pneumonia or necrosis of muscles and organs such as liver, spleen or heart (DUBEY, 2010). The aim of the present study was determine the prevalence of antibodies to *T. gondii* in groups of zoo and circus animals from Southern Italy.

Materials and methods

Sera of 69 clinically healthy mammals of six families (Bovidae, Camelidae, Cervidae, Equidae, Felidae and Hominidae) were collected in three zoos and four circuses in Southern Italy. The animals were divided into groups according to different criteria that are summarised in table 1. Every blood sample was centrifuged; serum was removed and frozen at -20°C until tested. Antibodies to *T. gondii* were

detected by indirect fluorescence antibody test (IFAT) using a commercially available *T. gondii* antigen IFR and anti-IgG FITC conjugates: anti-bovine and anti-goat IgG (VMRD, Pulman, USA) for Bovidae, anti-camel IgG (VMRD) for Camelidae, anti-deer IgG (KPL Inc. Gaithersburg, Maryland, USA) for Cervidae, anti-horse IgG (VMRD) for Equidae, anti-cat IgG (Sigma Aldrich, St. Louis, USA) for Felidae and anti-human IgG (VMRD) for Hominidae. The sera were diluted with physiological solution two-fold starting at 1:50; samples with titre ≥ 50 were considered positive.

Tab. 1: Characteristic of zoo and circus animals and seroprevalence of *Toxoplasma gondii*.

Characteristic	Total	IFAT positive (%)
Family		
Bovidae	10	2 (20 %)
Camelidae	23	7 (30.4 %)
Cervidae	2	2 (100 %)
Equidae	8	0 (0 %)
Felidae	22	21 (95.5 %)
Homidae	4	2 (50 %)
Gender		
Male	43	22 (51.2 %)
Female	26	12 (46.2 %)
Age (years)		
0 \leq 4	29	13 (44.8 %)
$\geq 5 \leq 7$	20	11 (55 %)
$\geq 8 \leq 15$	11	6 (54.5 %)
≥ 16	9	4 (44.4 %)
Country of birth		
France	2	1 (50 %)
The Netherlands	2	1 (50 %)
Hungary	1	1 (100 %)
Italy	28	15 (53.6 %)
Romania	4	2 (50 %)
Spain	19	12 (63.2 %)
Not known	13	2 (15.4 %)
Where they are bred		
Zoo	26	9 (34.6 %)
Circus	43	25 (58.1 %)
Housing		
Alone	2	0 (0 %)
Couple	5	2 (40 %)
Group	37	20 (54.1 %)
Not known	25	12 (48 %)
Contact with cats		
Yes	53	24 (45.3 %)
No	16	10 (62.5 %)
Total	69	34 (49.3 %)

Results and discussion

Antibodies to *T. gondii* were found in 34 (49.3 %) of all zoo and circus animals from Southern Italy with titers ranging from 50 to 3200. Animals from circus (58.1 %) were considered more positive than animals from zoos (34.6 %). *T. gondii* prevalence according to different criteria is summarised in table 1 and 2. The gender, age and kind of housing had no effect on prevalence. As expected, high prevalence (95.5 %) was found in Felidae with 92.9 % in *Panthera leo* (13/14) and 100 % in *Panthera tigris* (8/8). In the USA, CAMPS *et al.* (2008) detected lower *T. gondii* prevalence 54.5 % (12/22) and 27.8 % (5/18) in these two felids, respectively. The most common way of infection for felids is consumption of meat infected with *T. gondii* tissue cysts. This is in contrast with herbivores that are mainly infected through drinking water and with *T. gondii* oocysts contaminated food. We detected relatively low prevalence in herbivores (0 – 30.4 %). Similar *T. gondii* prevalence as in our study was found in families of Bovidae (20 %) and Camelidae (26 %) in zoos in the Czech Republic (SEDLÁK and BÁRTOVÁ, 2006). According to DUBEY *et al.* (2010), some primates are very sensitive to *T. gondii* infection. In present study, we detected *T. gondii* antibodies in two of four Hominidae that was lower compared to 73 % in Hominidae from zoo of the Czech Republic (SEDLÁK and BÁRTOVÁ, 2006).

Tab. 2: Characteristic and prevalence of *Toxoplasma gondii* in each family.

	Bovidae	Camelidae	Cervidae	Equidae	Felidae	Homidae
Gender						
Male	0/4 (0 %)	3/14 (21.4%)	2/2 (100 %)	0/5 (0 %)	16/17 (94.1 %)	1/1 (100 %)
Female	2/6 (33.3 %)	4/9 (44.4 %)	-	0/3 (0 %)	5/5 (100 %)	1/3 (33.3 %)
Age						
0 ≤ 4	0/1 (0 %)	5/14 (35 %)	2/2 (100 %)	0/6 (0 %)	6/6 (100 %)	-
≥ 5 ≤ 7	2/5 (40 %)	0/3 (0 %)	-	0/2 (0 %)	9/10 (90 %)	-
≥ 8 ≤ 15	0/1 (0 %)	2/6 (33.3 %)	-	-	4/4 (100 %)	-
≥ 16	0/3 (0 %)	-	-	-	2/2 (100 %)	2/4 (50 %)
Where they are bred						
Zoo	1/7 (14.3 %)	3/11 (27.3 %)	2/2 (100 %)	0/1 (0 %)	1/1 (100 %)	2/4 (50 %)
Circus	1/3 (33.3 %)	4/12 (33.3 %)	-	0/7 (0 %)	20/22 (95.2 %)	-
Contact with cats						
Yes	2/10 (20 %)	5/17 (29.4 %)	-	0/8 (0 %)	17/18 (94.4 %)	-
No	-	2/6 (33.3 %)	2/2 (100 %)		4/4 (100 %)	2/4 (50 %)
Total	2/10 (20 %)	7/23 (30.4 %)	2/2 (100 %)	0/8 (0 %)	21/22 (95.5 %)	2/4 (50 %)

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ASSESSING ANIMAL WELFARE IN A FLOCK OF NORTHERN BALD IBIS (*GERONTICUS EREMITA*) HOSTED IN CAPTIVITY FOR REINTRODUCTION PURPOSES

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Northern bald ibis (*Geronticus eremita*) is an endangered species almost disappeared in nature; the decline of wild population is mainly due to hunting, reduction of habitat quality and urbanisation that hinder the reproduction. For these reasons, Northern bald ibises are involved in a conservation project (Life + Reason for Hope) to ensure the survival of this species in nature.

The aim of this study was to evaluate animal welfare in a flock of 14 Northern bald ibises (seven adults and seven young individuals) hosted at Parco Natura Viva - Garda Zoological Park, Italy.

Duration of individual behaviour (e.g. preening, foraging, feeding and sunbathing etc.) and social behaviours (e.g. greetings, social preening agonistic interactions etc.) were collected using focal animal continuous sampling method. Twenty 10-min sessions per subject were run. Data were analysed using non parametric tests with the significance level set at $P < 0.05$.

Results of the study showed that the ibises performed species-specific individual (e.g. preening, foraging, feeding, exploration, sunbathing and others) and social behaviours (e.g. greetings, social preening and agonistic interactions). Moreover young subjects displayed significantly more individual behaviours than adults; in particular, exploration was performed more by juveniles than adults. On the contrary adult ibis spent more time resting than young birds. In addition, among social behaviours, agonistic interactions were performed more by the juveniles than adults whereas social preening was only displayed by adults.

In conclusion, results from this study highlight a good welfare of the ibis captive colony since the birds performed species-specific behaviours and did not show locomotor stereotypies or abnormal behaviours (e.g. feather damage, excessive aggression); moreover, the reported differences between adult and juvenile behavioural patterns could be considered in conformity with species-specific behaviours and Northern bald ibis developmental biology. These findings seem to suggest that the Parco Natura Viva ibis colony could be involved in reintroduction projects.

ELECTROCARDIOGRAPHIC REFERENCE INTERVALS IN HEALTHY ADULT WILD BORN CHIMPANZEES (*PAN TROGLODYTES*)

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Wild-born apparently healthy adult chimpanzees ($n = 118$) were anaesthetised for completion of routine health checks, using one of three different anaesthetic protocols (medetomidine (Domitor[®], Pfizer Animal Health, Exton, PA, USA) 0.05 mg/Kg ketamine (Vetalar V[®] Zoetis Ltd., London, UK) 5 mg/Kg; tiletamine-zolazepam (Telazol[®] Zoetis Inc., Kalamazoo, MI USA) 10 mg/Kg or tiletamine-zolazepam 2 mg/Kg in combination with medetomidine 0.03 mg/Kg). Body weight for all the animals was estimated based on visual body condition or previous recordings. Animals anaesthetised with ketamine-medetomidine were hand injected due to sanctuary preferences, the rest of them were darted. Twelve lead ECG was collected from each animal. Electrode position was defined according to that proposed in humans. Briefly; limb electrodes were attached to the respective wrists and ankles. Precordial electrodes were placed as follows: V1 and V2 were positioned in the fourth intercostal space on the left and right sternal borders respectively. V3 was placed between V2 and V4. V4, V5 and V6 were positioned on the mid-clavicular, anterior axillary and mid-axillary lines respectively, in the fifth rib space. ECGs were analysed for: Heart rate, P wave duration, PR interval, QRS duration, QT interval, QTc (Bazett) and QRS axis. Data were grouped by sex, and checked for normality using the Kolmogorov-Smirnov test. Differences between males and females were assessed using independent T-tests. ECG reference ranges for adult male (mean age \pm SD, 22 \pm 4) and female (mean age \pm SD, 18 \pm 6) chimpanzees are presented in table 1. There were significant differences between male and female chimpanzees in all variables other than P wave duration and PR interval. Future studies should set ranges for younger animals, influence of the anaesthetic protocols already used (more individuals in each group are needed), and combine ECG data with cardiac structural imaging.

Tab. 1: ECG reference intervals in adult wild born male and female chimpanzees.

Parameter	Mean \pm SD		5 th - 95 th Percentile		Mean \pm (1.96*SD)	
	Males	Females	Males	Females	Males	Females
HR	60 \pm 13	74 \pm 17*	37 - 80	52 - 103	35 - 85 [#]	41 - 107
P (ms)	99 \pm 12	91 \pm 11	80 - 126	69 - 108	76 - 122 [#]	70 - 112
PR (ms)	162 \pm 27	152 \pm 25	126 - 216	115 - 203	109 - 215 [#]	103 - 201
QRS (ms)	86 \pm 9	74 \pm 9*	69 - 102	60 - 88	68 - 104 [#]	56 - 92 [#]
QT (ms)	384 \pm 43	366 \pm 31*	342 - 463	314 - 424	300 - 468	305 - 427 [#]
QTc	380 \pm 28	401 \pm 31*	343 - 438	349 - 459	325 - 435 [#]	340 - 462
QRS axis	69 \pm 16	60 \pm 20*	21 - 85	11 - 82	38 - 100	21 - 99

* indicates significant difference from males ($P < 0.05$), [#] indicates normally distributed data.

ULTRASONOGRAPHY AS A CONFIRMATORY SEXING METHOD IN RETICULATED PYTHONS (*MALAYOPYTHON RETICULATUS*)

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Summary

Despite having a wide geographic range and being a common species both in the wild and in captive collections, field methods of sexing have not been investigated in adult reticulated pythons (*Malayopython reticulatus*). We compared two methods of sex determination: i) hemipenal probing and ii) ultrasonography. We found hemipenal probing to be inadequate as a decisive sexing technique due to the ambiguity of the large range of probing depth which overlapped in both sexes in the range of 14 to 17 scales. Instead, we recommend ultrasonography as a confirmatory technique when hemipenal probing presents ambiguous sexing results of adult reticulated pythons.

Introduction

Determining sex in reptiles has led to the development of a variety of techniques since many reptile species do not exhibit a distinct sexual dimorphism. These techniques include hemipenal eversion (MAYES *et al.*, 2005), morphometric ratios (FOWLER and SALOMÃO, 1995), ultrasonography (GNUDI *et al.*, 2009) and hemipenal probing (MARAIS, 1984; FITCH, 1987).

Hemipenal probing has been an established method of sex determination for snakes since the 1930s (SCHAEFER, 1934) and is commonly used in captive reptile husbandry. The technique is performed by inserting a blunt probe ventrally into the cloacal opening until it cannot be further inserted, indicating the end of the hemipenal pocket (FITCH, 1987).

For most species of snakes, the probe can be inserted to a depth of "seldom more than three sub-caudal scales" for females and eight to 16 scales for males (MARAIS, 1984). This is because females lack elongated two-pronged hemipenal pockets which males possess. Similarly, husbandry manuals for pythons report ranges of three to five scales in females and 10 - 16 in males (BARTLETT and WAGNER, 1997; DE VOSJOLI and KLINGENBERG, 2012). However, there have been ambiguities found in the method of hemipenal probing, as found in the invasively dissected *Boiga irregularis* on Guam where the authors found probing under field conditions to be an inaccurate sexing method (JORDAN and RODDA, 1994).

We observed some inaccuracies in the probing method for reticulated pythons when ultrasound examinations performed on an *ad-hoc* basis (e.g. for diagnosis) and opportunistically detected ovaries were detected in snakes that had been probed to be male. From these first scanning experiences we found that hemipenal probing was decisive when the probing depth was > 18 scales (i.e. male) but ambiguity appeared for snakes probed < 17 scales. In contrast, ultrasonography has been found to have 100 % accuracy (GNUDI *et al.*, 2009). Therefore, in this study we investigate the efficacy of hemipenal probing as a sexing method in adult reticulated pythons, using ultrasonography as confirmatory method.

Materials and methods

Wild reticulated pythons found in human-animal conflict situations are brought to Singapore Zoo for general health checks as part of an ongoing mark-recapture study. As part of data collection, all adult reticulated pythons (snout-vent length > 200 cm) were sexed using hemipenal probing.

Prior to the hemipenal probing and the ultrasound examination, pythons were sedated with Zoletil® (5 - 10 mg/kg, Tiletamin-Zolazepam, VIRBAC, Fort Worth, TX, USA) intravenously injected into the left palatine vein. After 3 - 5 min a 15 cm straight sexing probe (0.096 inch diameter), lubricated with K-Y Jelly® gel (Johnson & Johnson®, New Brunswick, NJ, USA) was gently inserted at the caudo-ventral opening of the cloaca. The probe was oriented towards either the right or left hemipenal pocket and gently eased in until it could not be further inserted. The inserted probe-length was marked and held against the ventral scales of the tail to obtain the scale count.

Ultrasonography was conducted using a portable ultrasound machine (Esaote, MYLAB30Vet Gold, Genoa, Italy) equipped with a 3 - 9 MHz transcutaneous convex probe. The snakes were scanned under water to provide better coupling.

Results and discussion

In 2014, 354 wild caught adult reticulated pythons were sexed using hemipenal probing. Selected adult individuals ($n = 92$) probed to have a scale depth of < 17 (see figures 1a and 1b) subsequently underwent reproductive ultrasound examinations. Using the probe in sagittal position, the liver which is easily identifiable in cross-section was followed caudally until its image is taken over by the stomach (figure 2). Following the stomach caudally, the position of the gallbladder indicates the beginning of the duodenum, which corresponds to the location of the cranial portions of the right gonad (figure 3). At this point the probe is adjusted into linear position to visualise the right gonad, which is located anterior of the left gonad. When viewed longitudinally, ovaries in snakes appear like a pearl necklace (figure 4), which depending on the cycle status varies in diameter sizes of a few millimetres to several centimetres. An individual with a scale depth of 13 scales was found to be male as ultrasonography revealed the presence of testicles (figure 5). Pre-reproductive ovaries and inactive testicles (outside of breeding season) were more difficult to detect and differentiate. However, all female snakes scanned in this trial were of body sizes (snout vent length > 200 cm) in which the presence and absence of ovaries could be clearly determined.

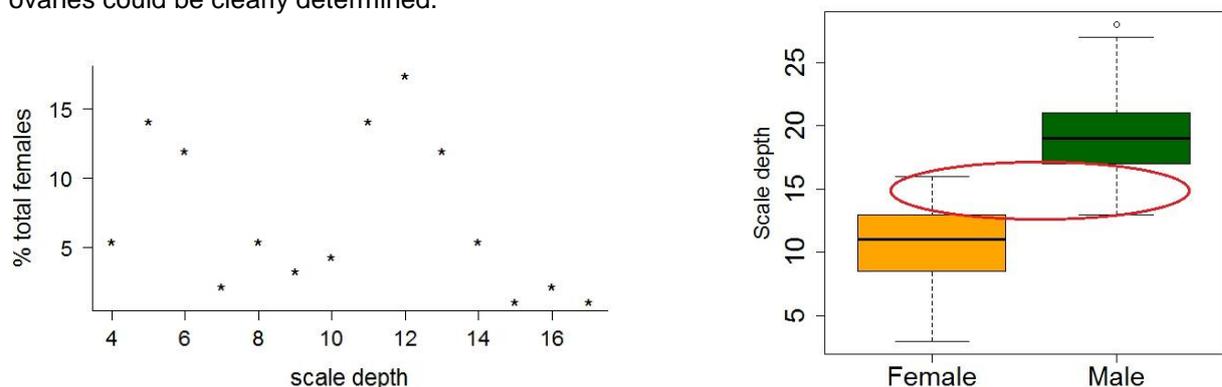


Fig. 1: a (left): Percentage of total female snakes ($n = 92$) confirmed with ultrasonography in relation to their corresponding hemipenal pocket scale depth. (© Wildlife Reserves Singapore)
b (right): Hemipenal probing scale depth of males ($n = 207$) and females ($n = 147$), of which 92 females were selected for verification by ultrasonography. The red circle indicates the overlap of scale depth between the two sexes. (© Wildlife Reserves Singapore)

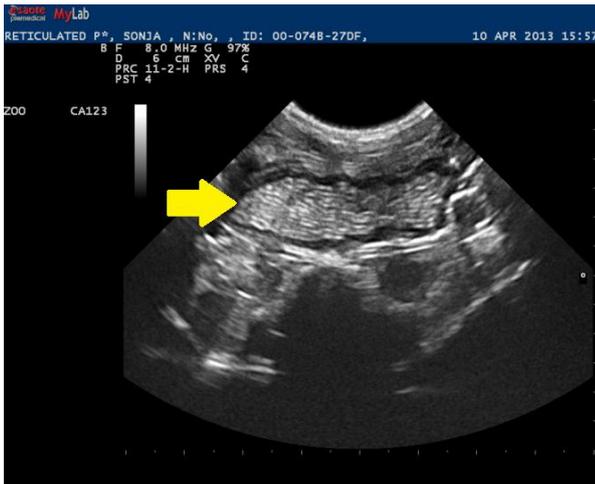


Fig.2: Ultrasound: Stomach in cross section.
(© Wildlife Reserves Singapore)

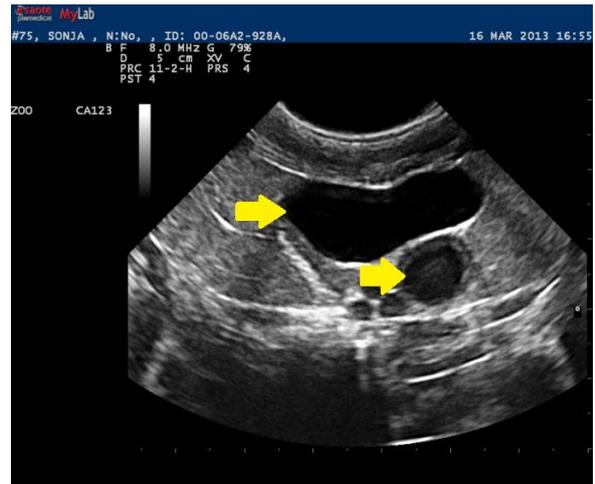


Fig. 3: Ultrasound: Gallbladder (arrow above)
with follicles of right ovary (arrow below).
(© Wildlife Reserves Singapore)



Fig. 4: Ultrasound: Pre-ovulatory follicles.
(© Wildlife Reserves Singapore)

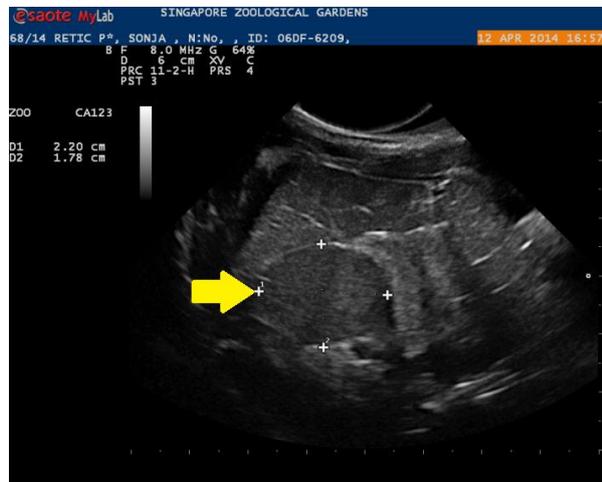


Fig. 5: Ultrasound: Male testicle in cross section.
(© Wildlife Reserves Singapore)

Here, we compared the efficacy of high-resolution ultrasonography versus traditional hemipenal probing for obtaining verifiable and accurate sex determination data in the reticulated python. While our data and results are only focused on one species, we advise caution to any researcher trying to accurately determine sex of snakes solely based the method of hemipenal probing. We therefore suggest using conservative criteria and to account for ambiguity and overlap in probing scale depth. The values of probe depth should be clearly stated in studies in which the sex of the individual is an important datum.

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CAPTURING AN OLD, PROBLEMATIC PERSIAN LEOPARD (*PANTHERA PARDUS SAXICOLOR*) IN IRAN-TURKMENISTAN BORDERLAND

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Summary

A Persian leopard was known to attack domestic animals near a village, named Tazeh Ghaleh in north-eastern Iran. During summer, mainly small livestock were killed. Later, the leopard switched its predation to herd dogs and attacked three people causing severe injuries (they recovered after one month). In October 2014, the villagers reported the situation to the local authorities, seeking for a solution to stop losing animals. The Iran Department of Environment (DoE) was not successful, so it asked the authors to help them. Foot-snares were set at three locations, all within the village's buffer zone. Remote monitoring of snares was achieved by fitting a Telonics TBT-500 trap site transmitter to them. On day three, the snares were modified. Also, two more snares were added to the supposed route of the animal's trail based on camera trap footages. Later one of the newly deployed traps was activated and the leopard was captured. Anaesthesia was safely achieved; leopard's biometrical statuses were measured and biological samples were taken. Dental structure revealed that it was an old adult male leopard, estimated more than 15 years old. Biochemical examination of the blood sample revealed severe hepatic and renal problems. It was concluded that instead of fitting the leopard with a GPS collar for translocation to a nearby reserve, the leopard needed intensive veterinary care and treatment. Therefore, the leopard was translocated to the Wildlife Rehabilitation Centre in Tehran for further treatment.

Introduction

The Persian leopard (*Panthera pardus saxicolor*), is the largest leopard subspecies. It is native to Iran, eastern Turkey, the Caucasus Mountains, southern Turkmenistan, and parts of western Afghanistan (figure 1). It is considered endangered with a population size of less than 500 mature individuals and a declining population trend (ZIAIE, 2008; KHOROZYAN, 2008).

For a long time, the Persian leopard has been widely hunted throughout its range. Nowadays, only fewer than 500 individuals live in west Asia and the majority of this endangered subspecies lives in Iran. Over the past 25 years, the leopard was exterminated in many areas of this vast range, and its number was significantly decreased in its other habitats. Surprisingly, the drastic decline of population size has been due to poaching, prey reduction and habitat loss. Lack of any direct actions focusing on

conservation of the Persian leopard in Iran has brought this cat to the verge of extinction (KIABI *et al.*, 2002).

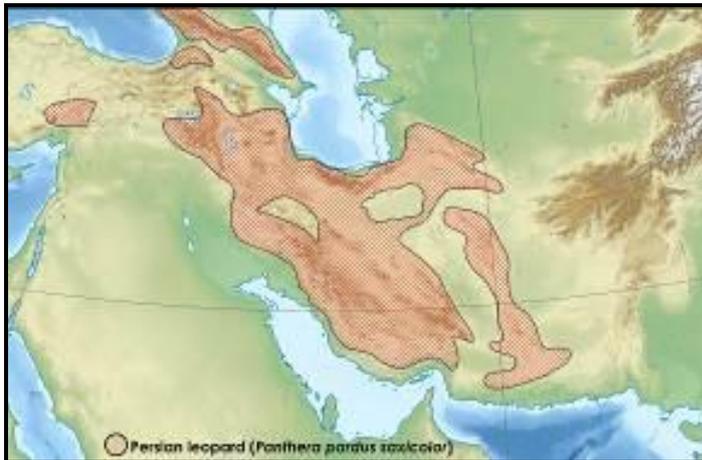


Fig. 1: Distribution of the Persian leopard (*Panthera pardus* spp. *saxicolor*) depicted as brown hatched area. (Image: Jannati E)

The major conflict between Persian leopards and humans is due to the traditional way of keeping livestock. The herders in Iran let their livestock graze anywhere, including national parks. In this situation, loss of livestock and herd dogs due to leopard attacks is inevitable. Hence, in the absence of any direct supervision from the government, herders kill leopards to protect their herds. In total, 63 % of the killing records from last year have been due to poaching or poisoning, and 22 % from road accidents. The provinces of Fars, Golestan and Lorestan have recorded the highest numbers of leopard poaching (MEMARIAN *et al.*, 2014).

Capturing and anaesthetising wild animals is an integral part of many wildlife research projects, particularly those involving cryptic, secretive animals inhabiting dense habitats where visibility is poor. Safe and effective techniques are essential, especially for endangered species (MIQUELLE *et al.*, 1999). Chemical immobilisation must rely on a safe drug, or drug combination, and an appropriate dose, which not only anaesthetises the animal, but also allows sufficient maintenance of physiologic processes such as respiration and heart rate (FAHLMAN *et al.*, 2011).

With a backbone of more than three decades, translocation of problem carnivores has been a standard method employed to remove them from areas of conflict (LINNELL *et al.*, 2007).

Case

Since August 2014, a Persian leopard was known to attack domestic animals near a village, named Tazeh Ghaleh in north-eastern Iran (figure 2). Located just a few kilometres from the Turkmenistan border, the village is surrounded by rolling terrains (figure 3) with juniper trees (*Juniperus* sp.) sparsely distributed on slopes within the Kopet Dagh region, one of the world's highest biodiversity hotspots.

During summer, small livestock such as sheep and goat were mainly killed, because most of the local herds spent overnight in the pastures. Since October, most herds concentrated their daily grazing around the village and were kept within properly-built corrals inside the village during night time. In an attack to one herd, dogs started following the leopard and the leopard preyed on one of them. Afterwards, the leopard switched its predation from livestock to herd dogs. Moreover, the leopard attacked three people when they tried to approach it on the killed dogs and caused severe injuries (they recovered after one month). As a result, people became frightened within the village and avoided to leave their houses overnight. The leopard track was detected after snowfall along the village's main routes and even its stool was found on one of the houses' roof.



Fig. 2: Location of Tazeh Ghaleh village in north-eastern Iran. Signs on the map denote to trap site (square), leopard track (star) and location of dog predation by the leopard (plus). Almost half of the attacks to the dogs occurred outside the village area. The leopard was captured at trap 3. (Image: Jannati E)



Fig. 3: View of Tazeh Ghaleh village, photographed from a ridge where the leopard was frequently seen. Turkmenistan's border is seen as snow-covered mountains beyond the village. (Photo: Farhadinia M)

In October 2014, the situation was reported to the local authorities, seeking for a solution to stop losing animals. Thus, Iran Department of Environment's experts tried to apply a variety of methods to resolve the problem by trying to keep the leopard away from the village. Besides provision of necessary information, particularly with respect to encountering a leopard in the wild, they tried to make fires around the village, turning gunpowder around high risk places, used aerial shooting to frighten the leopard, but all seemed not efficient to stop the leopard from killing dogs. Simultaneously, the DoE was under pressure from local and regional authorities to resolve the problem or to shoot the animal. Thus, translocation was considered in mid-December 2014 as the final solution which was based on the DoE's initial perception that it was a young "inexperienced" male.

Later the DoE asked the authors for help, since they had successful experience in capturing leopards. On reviewing a camera trap movie, it was revealed that they were facing an old male, not a young inexperienced one.

Between mid-August and mid-December 2014, the leopard killed 15 herd dogs (figure 4), which is equal to one animal per eight days. Also, a few sheep were reported to be killed by the leopard, but to the best of the author's knowledge none of them truly occurred. The area has a depleted level of prey, such as Urial sheep (*Ovis orientalis vignei*) and wild boar (*Sus scrofa davidi*), but they are unlikely to provide enough prey for the predator.

Foot-snares which have been successfully applied to capture leopards in Iran by the authors were deployed. As leopards are known to respond to baits, a dog killed by the leopard was used for the trapping. Traps were set at three locations, all within the village's buffer zone (figure 2). Each trap was composed of two sets of snares in order to increase capture chances. Remote monitoring of snares was achieved by fitting a Telonics TBT-500 trap site transmitter (Telonics Inc., Mesa, USA) to snares to signal the triggering of traps which were checked by radio-receiver at 30 - 60 min intervals. We informed the local people asking them to avoid trap sites as well as to keep their dogs indoors during night time to prevent any chance of dog predation during the trapping period. Also, we asked local authorities, especially border guards, to support us during the capture operation. So that during the capturing process, local inhabitants would not be present at the location.

The leopard came to the third trap site on night two (18:45'), but he was not snared due to non-apparent reasons. The trap was prepared again by the group and the leopard was spotted using

headlight at a distance of some 100 meters from the trap. He came back at 23:00', according to camera trap footage time stamp. He dragged the carrion on the snares, so the snares were not activated and he spent there until 05:00'(figure 5).

On day three (20 December), the snares were completely modified and the kill was tied to prevent easy dragging. Also, two more snares were added to the route where it was supposed to be the animal's trail. This was based on camera trap footages. At 17:00', one of the newly deployed traps was activated and the group arrived at the place within 20 min. The leopard was captured with forepaw and only three of his fingers were trapped inside the snare (figure 6).

The leopards weight was estimated 75 kg and anaesthesia was safely achieved using a combination of Ketamin 10 % (Alfasan, Woerden, The Netherlands) 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. The darting site was the triceps brachii (figure 7) (table 1).

Tab. 1: Mean of seven important parameters recorded from darting time to recovery.

^(A)The last measured temperature after induction and the first one respectively (measured every 10 min). ^(B)The last respiration rate and the first one counted during anaesthesia respectively (counted every 5 min). ^(C)The last pulse rate and the first one counted during anaesthesia respectively (counted every 5 min).

Parameters	Range	Mean
1. Induction time (min)	5	5
2. Temperature (°C)	37.8 - 38.2 ^A	38
3. Respiration rate/min	12 - 18 ^B	15
4. Pulse rate/min	77 - 80 ^C	78.5
5. Spo2 %	98 - 99	98.5
6. Head up time after Atipamazol inj (min)	6	6
7. Recovery time (min)	10	10



Fig. 4: The herd dog killed by the leopard and used as bait for the trap. (Photo: Shahrđari A)



Fig. 5: The leopard dragged the carrion on the snares, so the snares were not activated. You can find the inactivated snare and the leopard foot print in the photo. (Photo: Shahrđari A)



Fig. 6: The leopard was captured with forepaw and only three of his fingers were trapped inside the snare. As a first step the snare was removed and the snare site was checked carefully. (Photo: Shahrdari A)



Fig. 7: When the vet and the assistance arrived the leopard's hind limbs were hidden behind the bushes and the animal was so aggressive so *Triceps brachii* was chosen as darting site. (Photo: Shahrdari A)

Capture-related injuries included minor cuts and abrasions to the feet. The Leopard's biometrical statuses were measured (table 2) (figure 8) and biological samples were taken (blood, skin, mucosal swab and faecal sample). Mucous membranes were a bit pale and the hair coat was poor. The teeth were yellow with well-worn and broken canines and incisors. Most of the lower and upper incisors were missing and the mandibular bone was exposed. Mandibular and maxillary canine teeth had developed fractures with exposure of the pulp chamber. A discoloured tooth and an extra oral draining fistula were also found, which could indicate the presence of endodontic disease. Dental structure revealed that it was an old adult male leopard, estimated more than 15 years old (figure 9). Accordingly, it was concluded that instead of fitting the leopard with a GPS collar for translocation to a nearby reserve, the leopard needed intensive veterinary care and treatment, so there was not any chance of re-wilding this individual. Therefore, the leopard was translocated to the Wildlife Rehabilitation Centre in Tehran for further treatment.

Blood work revealed no abnormality in the CBC, but biochemical examination of the serum sample revealed functional renal problems (table 3), but the ultrasonographic examination showed no change in the echogenicity or structure of both kidneys and the IVP (intravenous pyelogram) showed a normal size, shape, and position of the urinary tract. The leopard was treated with Enalapril 5 mg (Enalapril 5-ABIDI, Tehran, Iran) 0.25 mg/kg PO once daily and IRC-VET (Pharmadiet VETERINARIA, Spain) 2 tablets PO once daily.

Tab. 2: Nine general parameters recorded about the captured leopard's status.

Parameters	Status
1. Age	More than 15 years
2. Sex	Male
3. Body weight (kg)	60
4. Head and body length (cm)	128
5. Tail length (cm)	85
6. Head length (cm)	30
7. Head cir (cm)	57
8. Neck girt (cm)	45
9. Shoulder (cm)	71



Fig. 8: The capture team investigating the anaesthetised leopard.
(Photo: ShahrDari A)



Fig. 9: The leopard's teeth were yellow with well-worn and broken canines and incisors which revealed that it is an old adult male leopard, estimated more than 15 years-old. (Photo: ShahrDari A)

Tab. 3: Eighteen general parameters including CBC and biochemistry tests of the serum sample.

Test	Result	Normal rang in cat
1. Hct %	38	29 - 45
2. Hb g/dl	10.1	8 - 14
3. RBC 102/ μ l	60	6 - 10
4. MCV fl	48	41.0 - 50
5. MCH pg	15.8	13.3 - 17.5
6. MCHC %	32	31 - 36
7. WBC 103/ μ l	16	5.5 - 19.5
8. Lymph %	22	20 - 56
9. Band %	1	0 - 3
10. Mono 103/ μ l	3	1 - 4
11. Eos %	10	2 - 12
12. Seg 103/ μ l	4097	2500 - 12500
13. AST/GOT IU/L	35	7 - 38
14. ALT/GPT IU/L	75	25 - 97
15. ALP IU/L	34	0 - 45
16. BUN mg/dl	30.46	20 - 30
17. Urea mg/dl	45.2	13.4 - 32.5
18. Scr mg/dl	2.68	0.8 - 1.8

Conclusion

The choice of any methodology for studying leopards depends on the purpose of the study, site location and the research team's experience and available resources. While leopard capture is still the most reliable methodology for biological sample collection and necessary for telemetry studies, due to the risks involved in these procedures, researchers tend to substitute them for non-invasive methodologies.

We do not encourage managers to consider live capturing of the problem animals in all similar situations, because studies from other leopard-range countries in Asia and Africa have concluded that translocation of 'problem' leopards is an ineffective and unreliable approach, and efforts should

instead focus on reducing the potential for conflict; most importantly, by improving livestock-husbandry practices (WEILENMANN *et al.*, 2010; ATHREYA *et al.*, 2011).

However, our attempt revealed that the impaired dental structure and severe systemic problems have made the leopard approach the village to kill domestic animals and despite extensive local efforts, the animal stayed around for several months. Therefore, we believe that it is crucial to obtain reliable information and to explore underlying causes of intensive conflicts before making any decision. Besides careful investigation of conflict remains, camera traps with movie option can be hired at domestic animal's kills to evaluate the predator's physical condition, particularly when endangered predators are involved.

In case that live capturing is the only available resolution, especially due to social pressure, choice of proper methodology is critical. Foot-snares were effective for capturing the leopard. The drug combination and dose generated safe and efficient anaesthesia in this free-ranging leopard with clinically acceptable physiologic responses.

The presented case shows only one example of the conflict between humans and predatory animals. To succeed in the conservation of Persian leopard in this habitat, we need to have a short remedy and a long-term plan to address this issue. A short remedy for controlling the rapid loss of this elusive species is to engage the public and increase their awareness of this disaster. Another, less short-term plan, is the implementation of community-based conservation projects. The insurance for the herds attacked by Persian leopards could be another short-term remedy (MEMARIAN *et al.*, 2014).

Long-term plan for conservation of Persian leopards in this habitat is to find new solutions for keeping cattle from traditional methods to methods with more efficiency and less habitat loss. Participatory programmes are needed to revive the prey populations in this habitat, which lost at least 80 % of it (MEMARIAN *et al.*, 2014).

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DETECTION AND MOLECULAR CHARACTERISATION OF COMMON AVIAN VIRUSES IN PSITTACINE BIRDS IN NORTHERN ITALY

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Several viral diseases are known to cause severe illnesses in psittacine birds, such as avian polyomavirus (APV), avian bornavirus (ABV), and psittacine beak and feather disease virus (BFDV). Aim of this study was to detect the prevalence of these pathogens in companion parrots referred at the Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta from different areas; moreover a phylogenetic analysis will be carried out to characterise detected strains and to highlight the presence of different territorial distribution. Twenty-eight samples of different species of psittacidae were collected. DNA and RNA were extracted from several organs (brain, spleen, liver, kidney, intestine and heart) of psittacine birds submitted for *post mortem* examination due to unknown cause of death ($n = 23$) and from feathers or tracheal swabs submitted for *intra vitam* diagnosis of latent infection in asymptomatic birds ($n = 5$). Real-time PCR protocols were performed to detect the presence of BFDV and APV. Two end-point PCRs were used to assess the presence of different ABV genotypes. Fourteen out of 28 animals tested positive for BFDV, four birds had positive results for APV and ABV RNA was amplified in three parrots (table 1). Animals tested positive for APV were also tested positive for BFDV. The phylogenetic analysis on positive samples based on ~ 400 bp fragments selected in variable regions of the BFDV, APV and ABV genome is in progress. Results will be presented during the conference.

In conclusion, considering the percentage of samples that resulted positive for BFDV, APV and ABV in this case series (50 %, 14 % and 11 %, respectively), and taking into account the consequences of the infection, it seems always pivotal to test parrots for these viruses even if there are no clinical signs or *post mortem* findings clearly indicative of diseases.

Tab. 1: Results of the molecular analyses carried out during the study.

	BFDV	APV	ABV
positive	14	4	3
negative	14	24	25

ATOXOPLASMA SPP. IN BLUE-CROWNED LAUGHINGTHRUSH (*DRYONASTES COURTOISI*) ADULTS AND NEONATES

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Between 1996 and 2013, 71 blue-crowned laughingthrush (*Dryonastes courtoisi*) chicks, a critically endangered passerine bird, endemic to China, were born at Mulhouse Zoo, in France. None of them survived past one year and 82 % died between zero and six days old with unidentified cause and despite the attempt to establish an artificial breeding protocol.

Atoxoplasma spp., also known as systemic isosporis is a coccidian parasite that can infect several species of birds. Based on previous histological findings strongly suggestive of atoxoplasmosis in parent-raised chicks found dead in the aviary, adult birds were suspected to be infected with *Atoxoplasma* spp. and to transmit this disease to their offspring. Although there is no evidence in the literature for atoxoplasmosis infection in neonate, hand-reared birds, the hypothesis of congenital transmission of atoxoplasmosis has been proposed regarding the high mortality rate in these chicks despite the controlled and regularly cleaned environment where they were raised.

Treatment with 25 mg/kg body weight toltrazuril (Baycox[®] 2.5 %, Bayer AG, Leverkusen, Germany) was implemented in adult birds. Coprological examinations were performed before, during and after the treatment to quantify the parasite load in faeces. Polymerase Chain Reaction (PCR) assay was used to test blood samples from the adult and liver, lung, gizzard and kidney samples from ten chicks that died in 2013 and 2014 (between 2 and 20 days old) to detect *Atoxoplasma* spp.

Before treatment an average of 181 *Isospora* spp. oocysts per gram were found in the faeces of the adults. This number was reduced to zero, one week after the beginning of the toltrazuril treatment. Five of the ten chicks had some tissue samples positive for *Atoxoplasma* spp. in at least one of the three repeats of the atoxoplasmosis PCR.

The treatment with toltrazuril appears to allow a significant reduction of the parasite excretion. The PCR results suggest a transovarian transmission of *Atoxoplasma* spp. but further investigation is needed for confirmation.

This study was done in part fulfilment of the requirements for the degree of Master of Science in Wild Animal Health, University of London, 2013 - 2014.

MYCOBACTERIUM MARINUM INFECTIONS IN DOLPHINS – 20 YEARS OF CO-EXISTENCE IN THE DOLPHINARIUM OF KOLMARDEN, SWEDEN

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Skin infections in a group of captive bottlenose dolphins (*Tursiops truncatus*) caused by *Mycobacterium marinum* are described in terms of clinical history, bacteriology, diagnostics, pharmacology, therapy and occupational/human health aspects. The discovery of the almost pathognomonic skin granulomas in the Kolmarden dolphins caused by *M. marinum* dates back to the mid 90's. Despite repeated sampling, final proof was not obtained until 2005, when *M. marinum* was isolated from a fatal case of chronic generalised pyo-granulomatous panniculitis. Since then, multiple positive cultures have been isolated from skin biopsies. At present, only low grade or dormant infections with occasional localised reactivation are seen in all adult animals. In 2008, the first drug susceptibility tests became available and long-term anti-mycobacterial therapy was initiated based on these tests and pharmacokinetics studies of two antibiotics, Etambutol and Rifampicin. Initially a multidrug treatment regime was chosen with following dosages: Enrofloxacin 5 mg/kg SID (Baytril[®], Bayer, Leverkusen, Germany), Etambutol 15 mg/kg SID (Myambutol[®], Meda, Solna, Sweden) and Rifampicin 5 mg/kg SID (Rimactan[®], Novartis, Basel, Switzerland). Medication with Enrofloxacin discontinued after 39 to 62 days, whereas Etambutol and Rifampicin medication continued for a total of 335 to 576 days. Due to evidence of accumulation in the first two dolphins undergoing treatment, the dosing interval for Rifampicin was changed to q 48 h during the following course of medication in all animals. Mild to severe adverse effects from medication, ranging from in-appetence, over elevated liver transaminases, to one case of severe non-regenerative anaemia, were documented. Initially infections regressed and all treated animals turned culture negative during and following treatment, but total eradication could not be achieved long term, as positive skin biopsy cultures reappeared 9 to 12 months post treatment. In parallel, cases of related "swimming pool granulomas" were reported in staff in the late 90's. In recent years such infections have not been reported, most probably due to pool reconstructions and changes in the life support system. Two major environmental mycobacterial investigations were carried out in the dolphinarium in 2011, documenting the presence of the infectious agent in very low concentrations in the filter systems, as expected, but also and more unexpected, with incoming frozen fish. In conclusion, eradication of skin infections in dolphins caused by *M. marinum* by antibiotic measures alone has proven difficult and several questions in terms of subtyping, transmission, epidemiology, pharmacology and dolphin immunology will need to be addressed in the future.

DISEASE RISK ANALYSIS FOR SCHMALLEMBERG VIRUS IN ZOOLOGICAL COLLECTIONS

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Schmallenberg virus (SBV) is a livestock disease transmitted by *Culicoides* midges that was first described in the late summer of 2011 in cattle in Germany (GARIGLIANY *et al.*, 2012a), spread through Europe and arrived in the UK in early 2012 (AHVLA, 2012). The virus causes transient fever in adult ruminants (MUSKENS *et al.*, 2012), but can also cause abortions and foetal malformations (GARIGLIANY *et al.*, 2012b, HERDER *et al.*, 2012, PEPERKAMP *et al.*, 2012, VAN DEN BROM *et al.*, 2012).

In order for SBV to be a threat to ungulates in a zoological collection and cause clinical concerns, pregnant animals need to be exposed to infected vectors during the first half of their pregnancy (WERNIKE *et al.*, 2014). To qualitatively assess this risk, both a competitive ELISA and a plaque reduction neutralisation test (PRNT) were carried out on stored serum samples from three separate UK collections; SBV PCR was carried out on *post mortem* tissues from aborted or deformed neonatal exotic ungulates and on serum samples from Asian elephants (*Elephas maximus*) close to their approximate time of sero-conversion; neonatal deformities and abortions at ZSL Whipsnade Zoo between 2010 and 2014 were reviewed and vector capture and identification were carried out. In conclusion, SBV poses little risk to seasonal breeders and herds in which the management of the breeding male is possible.

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THE EFFECT OF THE MOLTING PERIOD IN THE MEASUREMENT OF FEATHER CORTICOSTERONE OF NORTHERN BALD IBIS (*GERONTICUS EREMITA*)

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Corticosterone (Cort), the main glucocorticoid in birds, is assumed to be a stress indicator and a potentially fitness predictor of individuals and even populations. The analysis of this hormone deposited in feathers is thought to provide an integrated measure of Cort levels mediated by the hypothalamic-pituitary-adrenal axis during the period of feather growth. During this season, feathers are highly vascularised and numerous compounds such as circulating Cort are deposited into the keratin structure. The objective of this study was to explore possible differences of feather corticosterone concentrations (FCC) between feathers from birds with complete and incomplete molt. Thirty Northern bald ibis (*Geronticus eremita*) were non-invasively sampled during the molting period. Interscapular feathers were taken in order to avoid damaging flight feathers and sex, age and morphological aspects of feather status were recorded. As pg Cort/mm feather has been described as the preferable unit and with the purpose of homogenising feather length between individuals, different numbers of feathers were used for each individual (varying from 3 to 6). Individuals were then classified into two groups according to their feather molting stage: completed and uncompleted. A methanol-based extraction technique and a competitive Cort Enzyme Immunoassay (EIA) were used to determine FCC. A linear model revealed no significant differences among sexes ($P = 0.76$) nor ages ($P = 0.24$). However, there were significant differences of Cort levels between feathers collected from individuals in different molting stages ($P < 0.05$). Concretely, results showed that individuals with completed molted feathers presented higher levels of Cort in comparison with individuals still in the process of molt. Such data provide evidence that FCC depend on the molt cycle and this must be taken into account in future field studies. Identifying and sampling completed molted feathers is highly recommended in order to reduce methodological interferences and ensure equal time periods in which Cort hormone is deposited.

CLINICAL AND HISTOPATHOLOGICAL ASPECTS OF THE ALOPECIA SYNDROME OF SPECTACLED BEAR (*TREMARCTOS ORNATUS*)

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In Ursids, alopecia is predominantly due to parasites. Very few cutaneous diseases are described in spectacled bears (*Tremarctos ornatus*). For several years, captive spectacled bears have shown extensive alopecia leading to complete nudity. Former studies have not managed to identify the cause of this alopecia. The goal of our study was to define clinical and histopathological features of the alopecia syndrome of spectacled bear, via clinical surveys and skin biopsies.

First, we studied 55 skin biopsies from 16 healthy spectacled bears without any skin lesion to define normal features of the skin in this species. In normal skin, the *stratum corneum* and epidermis are thin, collagen bundles of the dermis are extremely well organised and very few inflammatory cells are found. Hair follicles are compound follicles widely spaced from one another. Sebaceous glands are well developed whereas sweat glands are small. Those features are consistent across the body except in rubbing sites (rump, interdigital spaces), where epidermal hyperplasia can be present and should not be considered as lesions associated with alopecia.

Then, we studied clinical and histopathological aspects of the disease thanks to clinical surveys and 144 skin biopsies from 15 alopecic spectacled bears. Clinical surveys revealed that this syndrome consists in an acquired, slowly extensive alopecia, without alteration of general health. Seasonal fluctuations of alopecia were described in two bears and spontaneous recovering of alopecia appeared in two bears. A bilateral symmetry of alopecia was present in seven bears, and pruritus was described in four bears. Histopathological examination of skin biopsies showed orthokeratotic hyperkeratosis and acanthosis of the epidermis. In the dermis, a perivascular to diffuse inflammatory infiltrate was found, primarily composed of lymphocytes and eosinophils in various amount, associated with small numbers of histiocytes and plasma cells. A deep follicular atrophy below the isthmus was noted, as well as lymphocytic infiltrate of hair follicles. Multinucleated giant cells were found within the sheaths of hair follicles in seven bears.

These features lead to the hypothesis of an immune-mediated disease, with a cytotoxic lymphocytic reaction against the follicular sheaths (lymphocytic mural folliculitis) and epidermis by CD3+ T-lymphocytes. The eosinophils found in the infiltrate could have been recruited by T-lymphocytes via the secretion of interleukin 5 (IL-5). Immunohistochemistry and other stains need to be done to define the origin of the giant cells, to prove the secretion of IL-5 and to further phenotype the lymphocytes of the infiltrate. The recruitment of new cases of alopecia and a careful follow-up of current cases are necessary to refine the clinical and histopathological features of this syndrome and to establish a correlation between the clinical stage of the disease and histopathological findings. Further studies need to be done to identify triggering and promoting factors of this complex and probably multifactorial disease.

HAEMORRHAGIC STROKE IN A PYGMY HIPPOPOTAMUS (*CHOEROPSIS LIBERENSIS*)

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A 20 year old female pygmy hippopotamus (*Choeropsis liberiensis*) presented with acute onset of neurological signs. She was unable to stand, had muscle fasciculations, and horizontal nystagmus. Haematology and serum biochemistry demonstrated neutrophilic leucocytosis with a left shift, marked increases in creatinine kinase and lactic acid dehydrogenase. Her condition gradually improved with two weeks of intensive care involving a sling supporting her weight. By three months after onset, she only occasionally showed subtle proprioceptive deficits, including mild transient ataxia and dragging the feet for a few steps. Nineteen months after initial presentation, she was again acutely ataxic with several episodes of falling over; within two days the ataxia was completely resolved. Seventeen days later she was found dead. Necropsy revealed multiple haemorrhagic foci within the pons and medulla oblongata, chronic intervertebral disc disease (T4 to T14), and polycystic kidneys. *Post mortem* magnetic resonance imaging (MRI) of the head showed a focal lesion in ventral right cerebrum consistent with a fluid-filled cavity. Histopathology of the brain revealed acute multifocal haemorrhagic infarction and multiple regions of malacia of varied chronicity; multiple acute haemorrhages and multifocal Wallerian degeneration were present throughout the spinal cord. There was marked generalised medial arteriolar hyperplasia indicative of systemic hypertension, and chronic renal disease (glomerulosclerosis, multifocal interstitial lymphocytic infiltration and fibrosis, multiple cystic tubules). Five additional cases of central nervous system infarction (stroke) were identified in a retrospective review of 95 adult pygmy hippopotamus histopathology reports, suggesting this to be a common condition in this species.

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INCLUSION BODY DISEASE IN A REPTILE COLLECTION FROM ZOO DA MAIA, PORTUGAL

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Summary

The following study reports a survey for Inclusion Body Disease in a reptile collection from the Zoo da Maia, Portugal. This disease is known to occur in snakes of both the *Boidae* and *Pythonidae* families in private and zoological snake collections. In order to search for typical intracytoplasmic inclusions, blood smears from 43 snakes were performed, as well as an ultrasound guided biopsy in one of them. Nine animals were euthanised and subjected to necropsy examination and *post mortem* histological analysis. Ten animals presented a positive diagnosis for inclusions on blood cells with slight clinical signs, which suggest that snakes may retain subclinical infection, making them asymptomatic carriers. Although there is evidence that this disease is contagious, the aetiology and mode of transmission remain unknown, although it is believed that an arenavirus is involved and the bloodsucking snake mite *Ophionyssus natricis* is thought to act as a vector.

Introduction

Inclusion Body Disease (IBD) is a progressive and fatal disease that affects Ophidians, mainly members of the *Boidae* and *Pythonidae* families (CHANG and JACOBSON, 2010; HETZEL *et al.*, 2013), and its presence has been detected in private and zoological collections all over the world. The disease is mainly characterised by neurological and digestive lesions and secondary immunosuppression, often leading to secondary infections. Asymptomatic carrier specimens are also described. Histologically, intracytoplasmic inclusions can be found in blood cells and also in central nervous system (CNS), liver, pancreas and kidney (CHANG and JACOBSON, 2010; HETZEL *et al.*, 2013). The IBD aetiology is still controversial, despite the implication that there might also be a viral cause (retrovirus or arenavirus). Both the pathogenesis and epidemiology are still poorly understood, making this disease a true challenge to be overcome in disease prevention among reptile collections. The following study reports a survey for IBD on a reptile collection from the Zoo da Maia, Oporto, Portugal, between December 2013 and April 2014, due to the history of various clinical signs compatible with this disease shown by some of the animals.

Materials and methods

Forty three ophidian specimens (31 *Boa constrictors*, six *Python regius*, four *Python reticulatus*, one *Morelia spilota*, one *Python molurus*) of different ages (16 juveniles, 27 adults) were included in this study. The animals were evaluated regardless of the presence or absence of clinical signs compatible with IBD. After a first evaluation of blood smears, only ten animals presented inclusions on blood cells and progressed to the next phase of the study, with one of these animals subjected to an ultrasound guided biopsy of the liver, kidney and lung tissues for histological evaluation. This animal was kept alive, but isolated from the others, for posterior tissues biopsy in case of necessity. The remaining nine animals positive for inclusions on blood cells were euthanised and subjected to a necropsy examination to detect macroscopic alterations, and a histological evaluation of kidney, intestine, liver, pancreas, lung and CNS tissue samples was carried out.

The sedation protocol for blood collections was assured by tiletamine hydrochloride and zolazepam hydrochloride (Zoletil® 100, Virbac, Carros, France) association (4 mg/kg, IM), and by anaesthetic induction for the ultrasound guided biopsy, alfaxalone BP (Alfaxan®, Abbott Animal Health, Saint-Laurent, Canada) (10 mg/kg, IM). The blood samples were collected from 26 animals by means of intra-cardiac puncture, from eight animals using palatine vein puncture, and from nine animals by means of coccygeal ventral vein puncture.

The fixation and staining of the blood smears was effected using Hemacolor® (Merck, Darmstadt, Germany). Samples collected by ultrasound guided biopsy and those collected during necropsy examination were preserved in 10 % neutral buffered formalin and processed for light microscopy according to standard procedures. Paraffin sections (2 µm) were stained with haematoxylin and eosin. The euthanasia of the nine animals which tested positive in the blood smear for IBD was performed by an intra-cardiac injection of pentobarbital sodium (Eutasil® 200 mg/ml, CEVA Saúde Animal, Algés, Portugal) (86 mg/kg).

Results and discussion

In this study, blood smears were used as an initial differentiation test between positive and negative specimens searching for erythrocyte and leukocyte IBD inclusions. However, blood smears are low sensitivity diagnostic tests (CHANG and JACOBSON, 2010). Of the 43 specimens, ten showed inclusions in red and white blood cells (figure 1). Nevertheless, it is not possible to state that the absence of inclusions in the smears of the other 33 suspected animals implies the absence of IBD infection.

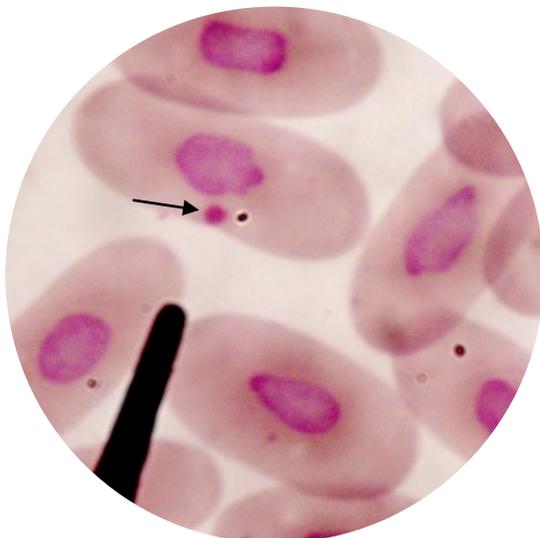


Fig. 1: Acidophilic Inclusion bodies in erythrocyte (Diff-Quik stain). (Photo: Alvura N, ©Zoo da Maia)

The *ante mortem* diagnosis should also be based on biopsies of the liver and oesophageal tonsil, tissues that usually present abundant intracytoplasmic eosinophilic inclusion bodies (CHANG and JACOBSON, 2010; HETZEL *et al.*, 2013). In this study, the animal subjected to ultrasound guided biopsy showed inclusions in the hepatic tissue, associated with the vacuolar degeneration of the hepatocytes, as previously stated (SCHUMACHER *et al.*, 1994; CHANG and JACOBSON, 2010).

The necropsy examination of the other nine animals showed slight cachexia, liver degeneration (light coloration of the liver) and pneumonia (figure 2), as described by CARLISLE-NOWAK (1998) and CHANG and JACOBSON (2010). One animal also showed signs of pneumonia and ascites, and in spite of these not being directly related to IBD, this latter symptom was previously reported (CARLISLE-NOWAK, 1998). In the optical microscope the inclusion bodies found match those described previously, of round to oval, 1 - 5 μm in diameter, intracytoplasmic eosinophilic inclusions (SCHUMACHER *et al.*, 1994; CHANG and JACOBSON, 2010). In the *Boa constrictor* species the inclusion bodies have mainly been found in the hepatocytes, epithelial cells of the renal tubules, neurons and glial cells of the CNS, and also in the pancreas and in the epithelial cells of the enteric tube and respiratory system (SCHUMACHER *et al.*, 1994). In this study, the major prevalence of inclusions was seen in the liver (all ten animals) and kidney, and particularly in the renal tubules in one of the animals. Other inclusions were also visible in the pancreas, intestine and lung (figure 3).

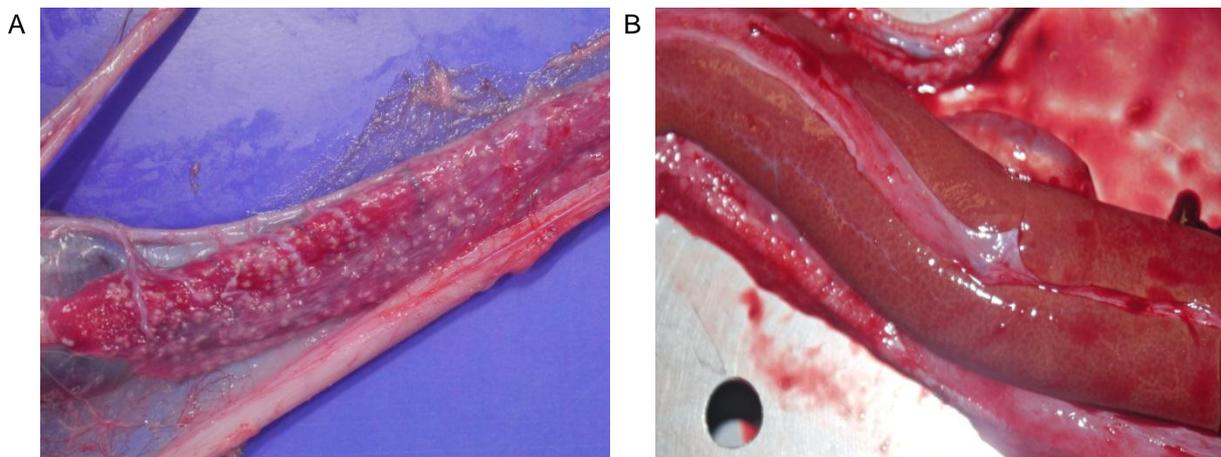


Fig. 2: A) Lungs with whitish foci – pneumonia. B) Light coloration of the liver - liver degeneration. (Photos: Pires I, Gama A, © Veterinary Hospital of University of Trás-os-Montes e Alto Douro)

IBD is probably the viral disease with the greatest preponderance in individuals from the *Boidiae* and *Pythonidae* families in captivity (CHANG and JACOBSON, 2010; HETZEL *et al.*, 2013). The aetiology of this disease remains controversial, the first study of IBD indicating the presence of a retrovirus as the suspected aetiological agent (SCHUMACHER *et al.*, 1994; WOZNIAK *et al.*, 2000; JACOBSON *et al.*, 2001; HUDER *et al.*, 2002). Nevertheless, most recently, the involvement of an arenavirus has also been suggested (STENGLEIN *et al.*, 2012; BODEWES *et al.*, 2013; HETZEL *et al.*, 2013). In this study, despite the presence of the two families, only the *Boa constrictor* species presented positive results. In the *Boa constrictor* the clinical presentation usually begins with food regurgitation followed by anorexia and cachexia (SCHUMACHER *et al.*, 1994), this being a wasting syndrome observed in one of the animals participating in this study. The fact that nine out of the ten *Boa constrictor* specimens with a positive diagnosis for IBD did not show particularly evident clinical signs, in spite of the necropsy observations, supports the documented possibility of subclinical infections transforming these animals into asymptomatic carrier specimens (CHANG and JACOBSON, 2010). There is evidence that IBD is contagious, though the means whereby it is transmitted remains unknown, although it is believed that

direct contact is involved. The bloodsucking snake mite *Ophionyssus natricis* is thought to act as a vector (SCHUMACHER *et al.*, 1994).

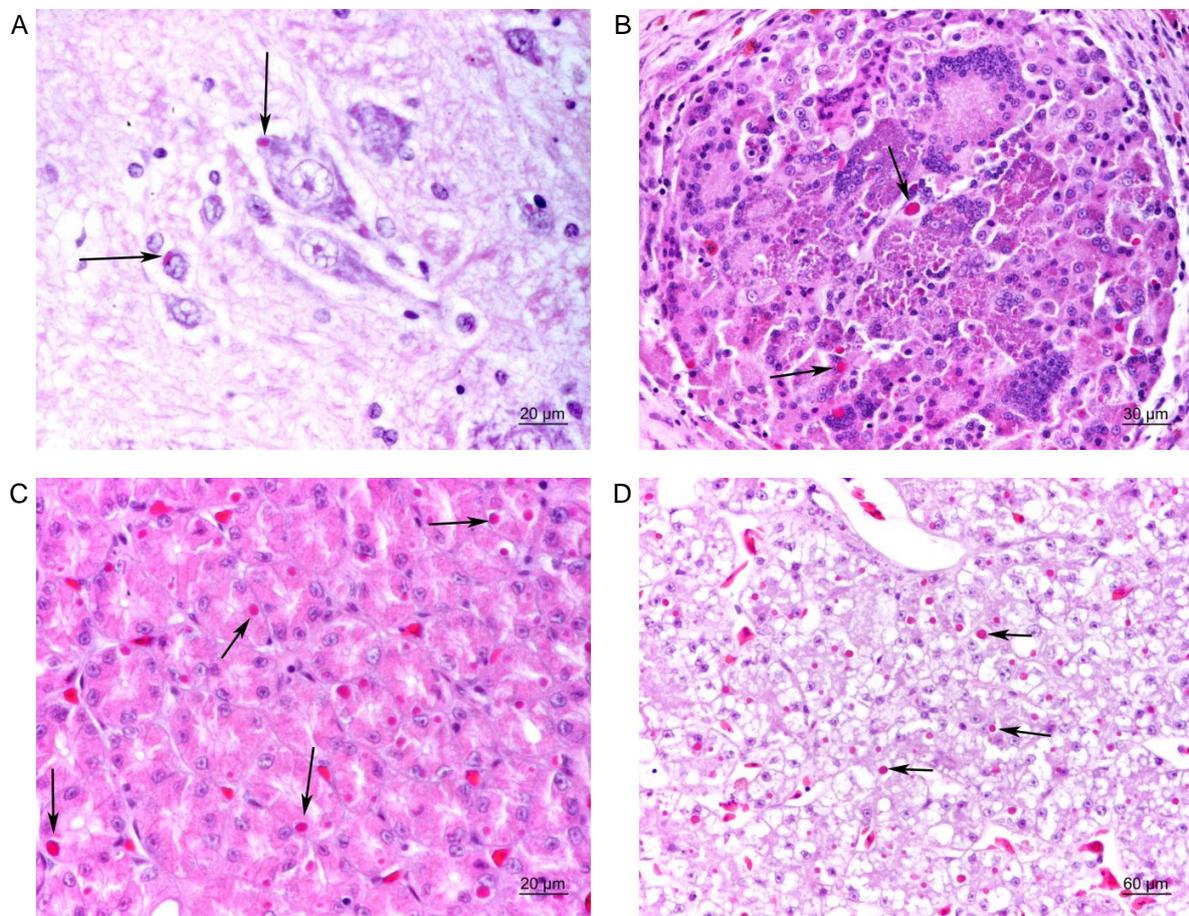


Fig. 3: A) Neuronal cytoplasmic inclusions. B) Acidophilic Inclusion bodies in lung granuloma lesion. C) Acidophilic inclusion bodies in kidney epithelial cells. D) Acidophilic Inclusion bodies in liver with lipidosis (all H&E). (Photos: Seixas F, Pires I, Gama A; © Veterinary Hospital of University of Trás-os-Montes e Alto Douro)

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EPIZOOTIOLOGICAL STUDY OF INSECTIVOROUS BATS IN SLOVAKIA

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The chiropteroфаuna is an important component of ecosystems in various types of landscape. Bats are commonly encountered in Slovakia where they use a variety of natural and seminatural habitats throughout the year. Ecological and epizootological monitoring and selection of research localities have been provided in the years 2013 - 2014 under licence of the Slovak Ministry of Environment in cooperation with members of the Slovak Bat Conservation Society and with National Slovak Nature Protection.

The public health risk in connection with potential European bat lyssavirus (EBLV) infection in bats was studied. Blood was collected for a serological survey on EBLV and presence microsporidia. Rapid fluorescent focus inhibition test (RFFIT) was used for the detection of specific rabies antibodies in the bats sera; no serological evidence for EBLV infection was observed. We also conducted a molecular detection of microsporidia in the group of captured bats. To identify *Microsporidia* spp. we used the method of real time-PCR; it has demonstrated the presence of *Microsporidia* spp. in 16 samples. In one sample *Encephalitozoon hellem*, and in four samples *Enterocytozoon bieneusi* have been detected. These species have a broad host specificity with a zoonotic potential.

For parasitological investigation, bat droppings were collected from wintering areas in the bats' caches and sampled during trappings. The bats' faeces were investigated for the presence of parasite eggs and protozoan oocysts using standard flotation methods. In total 88 faecal samples from bats belonging to 12 species were examined coprologically. The presence of parasites was ascertained in 64.60 % of specimens. Most frequent were *Eimeria* oocysts (61.36 %). In 9.09 % of the samples eggs of nematodes belonging to the suborder *Spirurina* and in 2.27 % eggs from the subfamily *Capillariinae* were detected. The hymenolepidid cestode eggs were present in two individuals (1.14 %) from one locality.

We studied the presence of ectoparasites on the bats. Three hundred and twenty eight ectoparasites were collected from 58 bats of ten species. We recorded bat ectoparasites of the groups *Acarina*: *Ixodidae* (*Ixodes verspertilionis* 1.72 %), *Macronyssidae* (*Steatonyssus occidentalis* 5.17 %) and *Spiritunicidea* – 92.13 % (*Spiritunnix mystacina* 79.32 % and *S. bechsteini* 20.68 %). From the order *Diptera* we recorded bat flies *Stylidia biarticulata* on seven bats; one species of fleas (*Ischnopsyllus intermedius*) was found on two bats.

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FIRST CONFIRMED CASE OF FATAL TUBERCULOSIS IN A WILD SRI LANKAN ELEPHANT (*ELEPHAS MAXIMUS MAXIMUS*) CAUSED BY *MYCOBACTERIUM TUBERCULOSIS*

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Tuberculosis (TB) is a major emerging infectious disease among captive elephants worldwide, and it is potentially zoonotic. To the best of our knowledge, no confirmed cases of TB have been reported in wild Asian elephants (*Elephas maximus*), and there are no confirmed cases of TB in captive or wild Sri Lankan elephants (*Elephas maximus maximus*). An emaciated 35 year old female wild elephant in moribund state was observed in Udawalawe National Park, Sri Lanka. The elephant was accompanied by a 1 1/2 years old female calf. The adult elephant died after two days of treatment with normal saline, dextrose, multivitamins, meloxicam (Melox 7.5MG, Alina Combine Pharmaceuticals (PVT.) LTD, Karachchi, Pakistan, 30 ml intravenously) and penicillin-streptomycin combination (Penstrep 400 LA, Interchemie, The Netherlands, 150 ml i.m.). Typical large caseous white nodular lesions were observed in >60 % of lung tissue during necropsy highly suggesting clinical tuberculosis. Samples from nodular lung lesions were decontaminated using Petroff's method and cultured on Löwenstein–Jensen (LJ) medium with glycerol. Pure culture acid-fast bacilli were isolated three weeks post inoculation. The isolate also grew on LJ medium with sodium pyruvate, but at a slower rate. No other bacteria or fungi were recovered on conventional media. The organism was identified as *Mycobacterium tuberculosis* based on culture characteristics and PCR for the presence of Genomic Region of Difference 9 (RD9). The source of the infection was unclear as no data is available on the human contacts of this elephant. Further there are no known wildlife reservoirs of *M. tuberculosis* in Sri Lanka. However we have previously observed three seropositive wild Sri Lankan elephants (unpublished data, using StatPakTB[®] and DPP[®] (Chembio Diagnostic Systems, Inc., Medford, NY, USA)). This is a classic case that demonstrates Asian elephants may maintain *M. tuberculosis* infection in the wild, and develop fatal tuberculosis.

FEATURES OF SPERMATOGENESIS IN DEER WITH TESTICULAR AND EPIDIDYMAL DISORDERS

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Although testicular cytology has been recognised as a reliable method for the assessment of spermatogenesis, its application in veterinary medicine is still limited. The aim of this study was to describe the features of spermatogenesis in three adult (> 4.5 years) red deer of unknown fertility affected by disorders of the reproductive tract. During the non-breeding season and from the same natural population, we found two individuals affected by epididymal cysts and one individual showing unilateral hypogonadism. Single and well-circumscribed epididymal cysts were located unilaterally or bilaterally in the caput epididymis of both stags. In order to provide a quantitative assessment of spermatogenesis, testicular samples were collected by fine needle aspiration cytology and stained with a Romanowsky-type stain. At least 200 spermatogenic and Sertoli cells were evaluated per each testis at 1000x magnification using light microscopy. Moreover, testicular mass and cauda epididymal sperm parameters were evaluated. Compared with normal values from adult individuals ($n = 6$) culled at the same date and location, epididymal cysts were associated with a low total epididymal sperm number (390.68 ± 188.27 vs. $1052.35 \pm 673.88 \times 10^6$ spermatozoa; mean \pm SD), high spermatogenic index (number of spermatozoa per 100 germ cells: 23.37 ± 0.03 vs. 16.13 ± 5.49 ; mean \pm SD), and a decrease of Sertoli cell functionality and workload indices (number of round spermatids per Sertoli cell: 2.21 ± 0.05 vs. 4.32 ± 2.10 ; germ cell number per Sertoli cell: 5.79 ± 0.75 vs. 10.27 ± 4.53 ; mean \pm SD), although differences were not statistically significant (Mann-Whitney U test, $P > 0.05$). Low total epididymal sperm number together with a high spermatogenic index might indicate sperm stasis, as a result of a physical obstruction to the normal progression of sperm cells. Cytology of the affected testis in the hypogonadic stag revealed a Sertoli cell-only pattern with morphologically normal Sertoli cells. The contralateral testis showed hypospermatogenesis (number of spermatozoa per 100 germ cells: 3.27), decreased Sertoli cell functionality and workload indices (number of round spermatids per Sertoli cell: 0.43; germ cell number per Sertoli cell: 2.83), and low total epididymal sperm number (261.19×10^6 spermatozoa), which may indicate testicular degeneration. This study shows for the first time how epididymal cysts affect spermatogenesis in red deer and highlights the role of testicular cytology as an effective screening tool of reproductive disorders in wildlife.

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ECHINOCOCCUS MULTILOCULARIS IN THE BEAVER (*CASTOR FIBER*) IN EASTERN AUSTRIA

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Alveolar echinococcosis (AE) has been present in Austria since 1854, and *Echinococcus multilocularis* is endemic in most regions of Austria. As people can get infected and develop severe disease, it is one of the most dangerous helminthal zoonosis and is fatal when untreated. Within a monitoring project of the European beaver (*Castor fiber*) population in eastern Austria several cases of AE were noted. In total, 398 (196 male, 202 female) European beavers were submitted for necropsy in the years 2009 - 2014. In six (1.51 %; three male, three female) of the animals multifocal to coalescing hydatid cysts surrounded by a dense capsule were seen in the liver. Samples were taken for histopathology, and the final diagnosis was made by PCR. All animals originated from Lower Austria, but no clustering could be seen. In histopathology the cysts were characterised by a whitish, chambered, multifocally mineralised capsule filled with a clear liquid. Microscopically these cysts were surrounded and separated by bands of fibrosis which extended into, disrupted and replaced the hepatic parenchyma. Many of the hydatid cysts contained several protoscolices. All except one animal did not show any other signs of disease. Due to the rabies-vaccination programme the fox population has been steadily increasing all over Austria, and an increase of *Echinococcus multilocularis* in foxes has been described by other authors. Unfortunately research has been limited to the western part of the country. The last thorough investigation in 2004 showed a slight increase of the parasite in foxes with a prevalence of 2.4 %. However it is assumed that the prevalence has been increasing in the last decade, but at the moment it is unknown for this area. The prior extinct European beaver was reintroduced in Austria 40 years ago. Since then the population has developed nicely, and nowadays can be found around the rivers Danube, Salzach and Inn. This inevitably has led to an overlap of the habitats of the two species and to an increased risk of transmission due to the faecal material of foxes containing the infectious eggs. Although only 1.51 % of the investigated animals were affected by this disease, the increased occurrence of this parasite in an intermediate host is of major importance, and highlights the importance of a continuous monitoring of the intermediate and final host species.

SUCCESSFUL TREATMENT OF *TRYCHOPHYTON RUBRUM* ON A PATAGONIAN SEA LION (*OTARIA BYRONIA*)

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Dermatophytosis is a superficial mycotic skin disease described in most mammalian species and because of its contagious and zoonotic potential it may causes substantial management issues in zoos. This disease is rarely seen in pinnipeds with only a few cases described in gray seals (*Halichoerus grypus*), harbor seals (*Phoca vitulina*), Steller sea lions (*Eumetopias jubatus*), Californian sea lions (*Zalophus californianus*), and Australian sea lions (*Neophoca cinerea*). These cases involved either *Microsporum gypseum* or *Trychophyton mentagrophytes*.

Here we describe a case of a 23-year-old intact female Patagonian sea lion (*Otaria byronia*) living in a group of three individuals (1.2) which presented multifocal to coalescing ulcerative skin lesions on the lumbar region. Skin scrapings were collected and a microscopic examination was conducted with no particular findings. A fungal culture was also realised and it revealed a *Trychophyton rubrum* infection. Oral terbinafine (Terbinafine Zentiva 250 mg, Sanofi-Aventis, Paris, France) approximately 2.3 mg/kg and topical enilconazole (Imaveral solution cutanée à diluer, Lilly France, Suresnes Cedex, France) were used as a treatment for a period of 75 days and complete recovery was observed visually. No relapse was observed one year later. No other animal in the group was affected by the disease. Epidemiological analysis revealed a dermatophytosis case in one of the keepers of the carnivore section, which occurred a few weeks before the lesions were diagnosed in the sea lion. The keeper had lesions compatible with *T. rubrum* even if no culture was performed; he was the main trainer of the affected animal and was engaged in indoor sport practice which is considered a predisposing factor to *T. rubrum* because of the associate conditions of humidity and temperature.

Trychophyton rubrum is a major causative agent of superficial dermatophytosis and represents nearly 70% of all cases of dermatophyte infections in humans. It is, in fact, considered a common anthropophilic species. This dermatophytosis has also been described in domestic animals (cats and dogs) and transmission from man to animal has already been highlighted. In the present case the pathogen was not demonstrated on the suspected human source but epidemiology strongly suggests the occurrence of an anthroozoonotic transmission.

To the author's knowledge this is the first description of a *T. rubrum* dermatophytosis case in a pinniped.

RETROSPECTIVE STUDY OF MORTALITY IN MALAYAN LESSER MOUSE-DEER (*TRAGULUS JAVANICUS*) AT BIOPARC FUENGIROLA

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During the last 12 years 20 *Tragulus javanicus* (Malayan lesser mouse deer) have been kept in Bioparc Fuengirola. Despite the good breeding success, the lifespan has been short and the mortality rate high.

Husbandry and pathology records of 11 male and 4 female mouse-deer that died between 2002 and 2014 were examined by cause of death and their relation to husbandry aspects: enclosure, season and diet.

The most common pathology finding (40 %) was a severe diffuse fat deposit and pancreatic acinar atrophy, leading to acute shock and sudden death in non-emaciated animals, similar to the peracute mortality syndrome described in giraffes. 13 % of the causes were trauma-related. All the other causes represented each one less than a 1 % of the mortality (one *Escherichia coli* septicaemia, one urinary bladder breakage due to calculus, one case of ruminitis, one dead in transit, one stillbirth and two animals with unknown death cause).

Comparing how husbandry influences mortality we found a higher number of deaths (67 %) in animals with lower energy and higher fibre diet. The season had also an influence, with 53 % of the deaths occurring during the four months with average temperatures lower than 15°C.

A negative energy balance could be suspected for the most frequent cause of death, as husbandry and dietary changes appeared to have reduced the mortality.

UROLITHIASIS IN ZOO ANIMALS – A REVIEW

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Urolithiasis is a common disease diagnosed by domestic animal veterinarians all over the world. The cause for the formation of urinary stones in domestic pets is complex. As a possible cause the diet plays a major role in addition to genetic factors such as congenital metabolic disorders. But certainly husbandry factors such as lack of exercise, lack of quantity or quality of drinking water supply or urinary retention are significantly involved in the genesis of urinary calculi.

Anatomical structures are directly associated with the clinical manifestation of uroliths in ruminants. Especially males with a long, thin urethra such as in sheep and goats are predisposed to form obstructions in the proceccus urethralis or the flexura sigmoidea.

In zoo animals, stones have been described in the past again and again. In addition to the well-known problems in turtles, some mammal species such as the maned wolf or otters are regularly affected. But even in giraffes, dolphins, elephants, camelids and many other species uroliths were detected, primarily during pathology. As one part of a doctoral thesis, a short questionnaire was sent to the majority of European zoological institutions including the questions:

1 – Do you see stones (uroliths, gall or stomach stones) in your facility? Yes or No?

2 - Did you publish your case? Yes or No? If Yes, where did you publish it - could you be so kind and send me the citation?

3 - Did you change anything in the diet? Did you see any improvement? How?

The aim of the first part of this study was to evaluate the occurrence of urinary stones in zoo animals as the doctoral thesis is on the influence of nutrition on the formation of urinary stones in zoo animals. Approximately fifty Zoos answered the questionnaire so far. The results of this survey are presented and interpreted in comparison to the assumptions made in domestic animal medicine.

ANTIMICROBIAL ACTIVITY AND SPECTRUM OF CEFOVECIN AGAINST BACTERIAL ISOLATES FROM MARINE MAMMALS

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Cefovecin is an extended-spectrum long-acting third-generation cephalosporin with an efficacy of two weeks after a single injection in dogs and cats. Different researches reported that animals of the order Carnivora, some Artiodactyla and bottlenose dolphins demonstrated protein binding levels > 99 %, and cefovecin pharmacokinetic researches in bottlenose dolphin (*Tursiops truncatus*), Patagonian sea lions (*Otaria flavescens*), and harbor seal (*Phoca vitulina*), revealed that elimination half-life and mean residence time were in all cases and dosages considerably longer than those previously reported for any other species.

On the basis of these facts the aim of this study is to evaluate the minimum inhibitory concentration (MIC) of cefovecin against some of the potentially pathogenic agents in marine mammals that are also present in dog and cat microbiota, where the drug is approved for use, to compare sensitivity and increase the knowledge about these values in bacteria from marine mammal species for a better understanding and use of this drug.

For this study, faeces, oral, nasal and ocular samples of six bottlenose dolphins (*Tursiops truncatus*), nine Patagonian sea lions (*Otaria flavescens*), eight harbor seals (*Phoca vitulina*), and three walrus (*Odobenus rosmarus*) were cultured for isolation and identification of *Escherichia coli*, *Clostridium perfringens*, *Staphylococcus aureus*, *Streptococcus* spp., and *Plesiomonas shigelloides*. MIC determinations against cefovecin were performed in duplicate by broth microdilution methods. The susceptibility of each isolate was determined using customised Sensititre microdilution plates (Nunc; Thermo Fisher Scientific, Roskilde, Denmark). The cefovecin concentration range tested was 0.06 to 64 µg/ml. Isolates were prepared for inoculation with cation-adjusted Mueller-Hinton broth (BBL, Becton Dickinson, USA), to which 5 % lysed horse blood was added for *Streptococcus* spp. The MIC range and mode results of cefovecin against bacterial isolates were as follows: range 0.12 - 64 µg/ml for *E. coli* (mode 0.5 µg/ml), range 0.12 - 8 µg/ml for *C. perfringens* (mode 1.0 µg/ml), range ≤ 0.06 - 2.0 µg/ml for *S. aureus* (mode 0.5 µg/ml), ≤ 0.06 µg/ml for both values mode and range for *Streptococcus* spp., and range ≤ 0.06 - 0.5 µg/ml for *P. shigelloides* (mode ≤ 0.06 µg/ml). This last bacterial species, *P. shigelloides*, was included in the study to show the effect of the drug on it as it is one of the most frequently cultured organisms in bottlenose dolphins (*Tursiops truncatus*).

The results suggest that cefovecin exhibits excellent in vitro activity across a broad spectrum of bacteria and its pharmacokinetic properties allow a less stressful treatment to exotic animals but it is necessary the continuation and expansion of this ongoing project including in the study clinical isolates to increase the reference values of MIC in these candidate species. A primary concern of this drug is about the need of a good adjustment of the optimal dose to minimise risk of appearance of resistant strains in those animals and the potential environmental and human implications based on the long lasting life of this critically important 3rd generation cephalosporin.

A CASE STUDY OF EMBRYO MORTALITY OF GREATER FLAMINGOS (*PHOENICOPTERUS ROSEUS*): STEPS OF AND REASON FOR EMBRYONIC DEATH

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Flamingos are monogamous birds and breed in dense colonies starting with a breeding display performed by the entire colony. In many zoos, mortality is 3.4 - 34.2 % and fertility 25 - 55 % depending on mixed or non-mixed colonies. Many studies have been focused on the difference between fertility and hatchability. However, nowadays there are very few researches focusing on flamingos' embryos and their development. The aim of this study was to investigate the embryonic development of non-hatching eggs of the first laying of a greater flamingos' flock in order to assess steps and possible causes of mortality. The embryonic development of *Gallus gallus* was used as model for flamingos. In 2013 the flock of 104 greater flamingos (*Phoenicopterus roseus*) housed at Parco Natura Viva – Garda Zoological Park, Italy, was observed. The embryonic observations of 17 non-hatching eggs of the first laying of the flock were analysed. Non-hatching eggs were measured and the development stage of embryo was observed. Results show that the shape index was 62.9 and the average of weight was 134.4 g. Embryonic observation show that seven embryos stopped developing before the organogenesis was complete whereas six embryos stopped developing after that process. Moreover four eggs were not fertile. Findings show that in greater flamingos as well as in *Gallus gallus*, non-hatching fertile eggs stopped developing near two critical periods: between stage 18 and 23 and between stage 44 and 46 of the *Gallus gallus* embryonic development model. Besides, as the embryo mortality occurred in all the eggs of the first laying, second egg-laying occurred. Overall the fertility of the 2013 season was high (88 %) even if hatchability was only 56.2 %. As in the 2013 breeding season precipitation occurred over the incubation period of the first-laying eggs whereas the level of rainfall was very low during the second period, an external factor (temperature and precipitation) might have been caused embryo mortality. In conclusion findings of this study seem to suggest that the embryonic development of *Gallus gallus* might be a good model to describe the development of flamingo embryos. Moreover, two periods of the embryonic development seem to be critical for the survival of the embryos. Furthermore, external factors as precipitation might impact the egg survival and the hatchling.

STUDY OF AN ANAESTHETIC PROTOCOL FOR VASECTOMIES IN VERVET MONKEY (*CHLOROCEBUS PYGERYTHRUS*)

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This study was undertaken to assess the reliability and safety of an anaesthetic protocol with multimodal analgesia during the vasectomy of Vervet monkeys (*Chlorocebus pygerythrus*).

The anaesthesia was performed on forty-five non-releasable vervet monkeys (*Chlorocebus pygerythrus*) with an estimated age of between three and eleven years. All specimens were healthy and lived in natural enclosures (minimum area of one hectare) with other males and females of their species at the Vervet Monkey Foundation (South Africa). They were unaccustomed to humans so at all times the contact with staff was minimised. The animals were trapped in cages designed to reduce stress during the process. Specifically, animals were attracted to the cages by food being left out in a refuge zone that was created inside the cage. Once an animal entered the cage, a rope system was employed so that contact with the subject was avoided. While still in the cages, the animals were then transported to the sickbay and kept there until they were re-introduced to their troop. In addition, eye contact between individual animals was avoided during their stay in the sickbay. The trapping cages were elevated from the floor and could be divided into two sections to allow cleaning and disinfection. The floor of the cages also acted as a squeeze panel to allow intramuscular injections. By reducing both visual and physical contact, and by also allowing intramuscular injections to be delivered quickly and easily, these cages minimised the stress on the animals. Anaesthetic doses were administered based upon estimated animal weight resulting in a mean drug combination of 7 ± 1.71 mg/kg of ketamine (Ketamidol[®], 100 mg/ml, Laboratorios Karizoo, Barcelona, Spain), 44.1 ± 7.85 µg/kg of medetomidine (Medeson[®], 1 mg/ml, UranoVet, Barcelona, Spain) and a fixed dose of lidocaine of 0.5 mg/kg (Lidocaina 2 %[®], B. Braun Medical, Barcelona, Spain) as local anaesthetic. During surgery, several physiological and cardiopulmonary parameters were recorded including: rectal temperature, heart rate, oxygen saturation, indirect blood pressure, capillary refill time, mucous membrane colour and the level of analgesic-sedation. To evaluate postoperative pain, feeding times were monitored for the presence of antalgic postures, bruxism, vomiting or vocalisations.

The anaesthetic protocol had a rapid induction time (between injection and loss of postural tonicity) of no more than 6 min and a mean immobilisation time (between loss of postural tonicity and first movement observed) of 41.06 ± 18 min. Vital parameters were within clinically acceptable limits and pain relief quality was shown to be optimal. However, fourteen animals (29 %) finished surgery with a body temperature lower than 37°C.

The protocol proved safe and provided enough analgesia for short-term surgical procedures such as vasectomy. Several animals displayed low rectal temperatures at the end of surgery, as expected, due to low body weight and high body surface in the species. Further studies should be carried out to investigate hypothermia prevention methods.

LEPTOSPIRA BRATISLAVA INFECTION IN A WILD IBERIAN WOLF (*CANIS LUPUS SIGNATUS*)

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Leptospirosis is a zoonosis caused by pathogenic spirochetes of the genus *Leptospira*, which affects most mammals. Leptospire can persist for long periods in kidneys of domestic and wild animal hosts, being shed through urine to the environment. We report a case of a sick yearling male Iberian wolf (*Canis lupus signatus*) that was admitted to the Exotic and Wild Animal Service, Veterinary Hospital of University of Trás-os-Montes e Alto Douro, Portugal, after being captured near a village in the Peneda-Gerês National Park, Portugal. Physical examination revealed cachexia, kyphosis, muscle atrophy of the hind legs and generalised alopecia with scabs all around the body surface. The animal was sedated to enable manipulation. During physical examination, marked icterus, low body temperature, weak pulse and tachycardia, as well as pain on palpation of the abdomen and lumbosacral area were relevant findings. Blood samples were collected for culture, haemogram, serum biochemistry and *Leptospira* serological testing. Urine was collected by ultrasound guided cystocentesis, observed under darkfield microscopy and used for urinalysis. Serum was analysed by microscopic agglutination test ("gold standard") with antigens of 17 different serovars of pathogenic *Leptospira*, revealing antibodies against serovars Bratislava (titre 1/600), Altodouro (1/200) and Icterohaemorrhagiae (1/200). Abortion and infertility are very common features in domestic and wild animals affected by leptospirosis. Serovar Bratislava is suspected of causing abortion in cattle and dogs. The current prevalence of antibodies anti-Bratislava in the wild boar from the Trás-os-Montes region is 6.9 %. These findings represent another potential threat to the conservation of the Iberian wolf.

ENDOCARDIOSIS OF RED FOXES (*VULPES VULPES*): AN ANATOMO-HISTOPATHOLOGICAL INVESTIGATION

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Endocardiosis is the most common cardiac disease in the dog and is closely associated with advancing age. As the disease progresses, there is destruction and derangement of the valve stroma with loss of collagen bundle organisation and accumulation of glycosaminoglycan in the leaflets, predominantly towards the valve edge. The cellular changes cause excess of proteoglycans, collagen loss and disorganisation in the valve stroma. Both structural and functional alterations of the valve are present, and a severe degree of endocardiosis can cause valvular insufficiency. Clinically, the degeneration of the valves and corresponding *chordae tendineae* causes an inappropriate coaptation of the leaflets leading to valve regurgitation and finally resulting in left sided congestive heart failure. In man, a morphologically similar disease has been described. In human myxomatous mitral valve disease (MMVD) causes prolapse or billowing of mitral valve leaflets into the left atrium during systole. The prevalence of MMVD in the human population is associated with age.

Fifty-five Red foxes (*Vulpes vulpes*) (35 males and 20 females), collected from different provinces of Piedmont (north-west Italy) were analysed by gross and histopathological investigation. Age class of the animals was determined by tooth eruption and degree of tooth wear (juveniles: $n = 5$; sub adults: $n = 19$; adults: $n = 31$). In all animals the heart was collected and fixed in 10 % neutral buffered formalin for gross and microscopical evaluation. The heart was examined following the inflow and out-flow tracts. On cutting open gross examination of the valvular leaflets revealed lesions of the tricuspid valve in 19 out of 55 samples (34.5 %), represented by endocardiosis ($n = 7$), valvular prolapse associated with endocardiosis ($n = 5$), thickening ($n = 3$), dysplasia ($n = 3$), and valvular retraction ($n = 2$); the latter two lesions may be interpreted as progression of endocardiosis. The histological pattern of the septal leaflet of the tricuspid valve showed the typical lesions of endocardiosis, characterised by mild, focal myxomatous degeneration extending into the complete valve, up to myxomatous transformation of the whole valvular leaflet. A peculiar lesion was represented by the transformation of the spongy zone of the valve into adipous tissue, associated with infiltration by mononuclear inflammatory cells. No lesions were detected in the mitral, semilunar aortic and pulmonary valves. Even if no statistical differences between age classes have been observed in the study, adult foxes appeared most affected and showed more severe lesions, although pathological signs of heart failure were not detected in the examined foxes.

Although based on a limited sample size, our investigation suggests the presence of endocardiosis in wild red foxes. Although in mammals endocardiosis is commonly detected in the mitral valve, according to our findings in red foxes the disease is prevalent in the tricuspid valve. Further investigations are needed to elucidate the aetiology and pathogenesis of these lesions in wild canid populations.

SURGICAL TREATMENT OF AN OLD HUMERUS FRACTURE IN A LOGGERHEAD SEA TURTLE (*CARETTA CARETTA*) - TREATMENT AND EVOLUTION

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The following clinical case report presents a female loggerhead sea turtle (*Caretta caretta*) weighting 25 kg with a shell fracture, presumably due to a boat collision. Additionally it presented a non-union fracture of the left humerus and another fracture at the right acromion.

The patient clinical and orthopaedic exam showed an absence of flipper movement, with stability loss while swimming to its left side. The radiographic and tomographic studies of both frontal flippers showed of a transversal diaphyseal fracture of the left humerus, and a transverse fracture of the right acromion.

After the functional evaluation of affected flippers, a surgical operation of left humerus in order to reduce and stabilise the fracture with osteosynthesis plates and the affected humeral fragments was performed. A medial approach was used exposing the left humerus. Once both fracture fragments were reduced it was then stabilised with locking osteosynthesis plates with 2.7 mm screws (SOP, Orthomed, Huddersfield, UK), both placed on the medial surface of the humerus. Initially a nine screws plate was placed, using 4 screws on the distal fragment and three screws on the proximal fragment. The second plate had seven holes where three screws were applied to the distal fragment and other two to the proximal fragment. After stabilisation it was applied to the fracture focus a human recombinant type 2 bone morphogenetic protein (rh-BMP-2) (Truscient[®], Zoetis, Berlin, Germany).

For 15 months the animal was submitted to a vary of different rehabilitation exercises (inside and outside of the water by means of passive flippers movement. There was no evident flipper autonomous movement until eight months after the surgery, when the movement increased slowly and gradually until the moment of its release where it was at 30 - 40 % of its functionality which was considered enough to the turtle survival in the wild.

The resolution of a humeral fracture using osteosynthesis plates, achieving a bone cicatrisation confirmed by radiography and a good flipper functionality after 18 months represents a new choice to the treatment of marine turtles with this kind of lesions.

We can then conclude that, the surgical resolution of a humeral fracture in a marine turtle using locking plates, and the application of bone cicatrisation promoters as rh-BMP-2, is a good alternative to flipper amputation.

HAEMODYNAMIC RESPONSE TO THREE DIFFERENT ANAESTHETIC PROTOCOLS IN WILD BORN CAPTIVE CHIMPANZEES (*PAN TROGLODYTES*)

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Blood pressure (BP) reference ranges for anaesthetised chimpanzees have been previously reported. However, these are based on a limited number of readings obtained early in a single anaesthetic protocol. Neither the BP response to different anaesthetic protocols, nor the haemodynamic time-course responses across a full anaesthetic procedure are known in chimpanzees. For the purposes of routine health assessments 155 apparently healthy wild-born chimpanzees were sedated using either: medetomidine (Dormitor[®], Pfizer Animal Health, Kent, UK) and ketamine (Kyron Laboratories, Benrose, Johannesburg, RSA) (MK) ($n = 101$, $M \pm SD$, age 13 ± 5 years, body mass 42 ± 16 kg); tiletamine-zolazepam (Telazol[®] Wyeth, Madison, NJ, USA) (TZ) ($n = 30$, $M \pm SD$, age 17 ± 12 years, body mass 32 ± 18 kg); or tiletamine-zolazepam and medetomidine (TZM) ($n = 24$, $M \pm SD$, age 16 ± 9 years, body mass 45 ± 19 kg). During each procedure heart rate (HR), systolic BP (SBP) and diastolic BP (DBP) were recorded every 3 - 5 min and the rate pressure product (RPP), a surrogate of myocardial work, was calculated. Data were grouped according to anaesthetic protocol and the mean value for the average HR, SBP, DBP and RPP over the course of the assessment was calculated. Mean (\pm 95 % confidence interval [CI]) haemodynamic responses were plotted against the time post-anaesthetic induction and presented graphically. Differences in mean data were assessed using Kruskal-Wallis tests, alpha was set at 0.05. Mean HR ($b \cdot \text{min}^{-1}$ [Confidence Interval (CI)]) was significantly higher in the TZ (86 [80 - 92]) trial compared with the MK (62 [60 - 64]) protocol, and was significantly higher in the TZM (73 [68 - 78]) protocol compared to MK. The average SBP and DBP (mmHg [CI]) were significantly higher in MK (130 [126 - 134] and 94 [91 - 97]) compared to TZ (104 [96 - 112] and 58 [53 - 93]) procedures and were also significantly higher in TZM (128 [120 - 135] and 88 [83 - 93]) compared to TZ protocol. Mean RPP was significantly greater in TZM (9368 [8805-9931]) than MK (8006 [7642 - 8370]). Time course data were visibly different between protocols, with TZM showing the steepest decline in HR, BP and RPP over the first 30 minutes and TZ showing the greatest fluctuation over time. The present data demonstrate that haemodynamics are highly variable dependent upon the anaesthetic protocol employed and the timing of measurement. Accordingly veterinary professionals should be cautious applying previously generated reference ranges to their specific population. The different haemodynamic time course data for each of the anaesthetics presented may enable clinicians to ascertain whether an observed response is "normal" under that specific protocol. The present data also highlight the need to obtain serial assessments instead of relying on single or duplicate measures to define BP in chimpanzees.

ABNORMAL ANTLER GROWTH IN TRUE HERMAPHRODITIC ROE DEER (*CAPREOLUS CAPREOLUS* L.)

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Summary

A 3 to 4 year old roe deer (*Capreolus capreolus* L.) was admitted to the veterinary teaching hospital after being hit by a car. The animal had female external genitalia: mammary gland, vagina, cervix and uterus, and carried well developed antlers with retained velvet. Internal sex organs consisted of a testicle and an ovotestis. Histologically ovarian tissue was functional but the testis did not produce sperm cells. External biometrical measurements were taken from the antlers and a CT exam was performed to evaluate its secondary sexual characteristics.

Introduction

Roe deer (*Capreolus capreolus* L.) have a low sexual dimorphism with males having deciduous antlers, although occasionally females can also develop antlers (MYSTERUD and ØSTBYE, 1999; FLIS, 2009).

Antlers grow by androgen influence, starting at a rapid growing stage with antlers covered with highly vascular velvet, proceeding to a second stage involving interrupted blood supply, a stage in which it was shedding the velvet, thus exposing the hard horn, and ending with a final stage in which the antlers are cast off (SEMPÉRÉ A and BOISSIN, 1981).

Retained velvet, sometimes combined with abnormal antler formation, is mostly seen in castrated bucks, but has also been reported in wild animals (BARTOŠ *et al.*, 2004). In female roe deer, antlers with retained velvet were usually associated with older animals. Stimulation by male hormones will not reach a sufficiently high testosterone peak for shedding the velvet (FLIS, 2009).

Material and methods/case description

In April 2014 a roe deer from Northern Portugal was brought to the veterinary teaching hospital of the University of Trás-os-Montes and Alto Douro, Vila Real, PORTUGAL, after being hit by a car. Despite treatment, it died from internal injuries and damage to the central nervous system.

Although the animal showed all the external characteristics of a female, a typical male antler formation with retained velvet was also present (figure 1A).

The roe deer was weighed and examined biometrically (body length, shoulder height, ear length and length of hind foot) using a tape measure with scale of 1 mm. Age was estimated from tooth wear (principally PM₃ and M₁).

A computed tomography (CT) scan was performed to skull and antlers (General Electric® GE Brivo CT325, 1 mm thickness sections with helicoidal acquisition).

A systematic *post mortem* examination was made within 3 hrs of death, with a collection of samples taken for histopathological examination. Specimens were fixed in 10 % neutral-buffered formaldehyde. The tissues were embedded in paraffin wax, sectioned at 3 µm and stained with haematoxylin and eosin (H&E).

Results

On external examination, the animal was considered an adult female, weighing 20 kg, with female external genitalia including vulvae and four teats. There was no external evidence of testicles or a penis. It was estimated to be between three and four years old through tooth observation: it had a two cusp third pre-molar, with M₁ still high and sharp. In this last tooth, the dentine had a half moon shape in both cusps (antero and posterolingual) and infundibulum (FERNANDEZ *et al.*, 2003).

Biometric measurements were: total length 123 cm; shoulder height 72 cm; ear length 12 cm and length of hind foot 29.5 cm.

The CT scan (figure 1B) revealed a bone antler equivalent of a second year male: pedicles, two main beams and tines. The length of the left main beam was 111 mm without tines and the length of the right was 116.9 mm, with two tines in posterior position. The longer tine was approximately 41.4 mm and the smaller tine 12.2 mm in length.

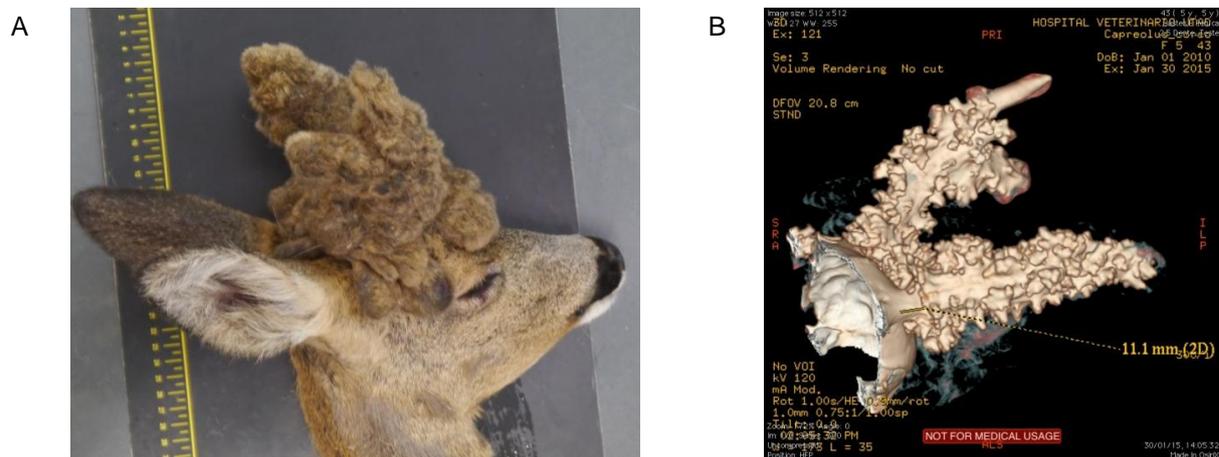


Fig. 1: Perruque; A) Macroscopic image; B) 3-dimensional CT image. (Photo A: Silva F; image B: Ginja M; both: ©Veterinary Hospital of University of Trás-os-Montes e Alto Douro)

Internal macroscopic examination revealed a complete female reproductive tract with vagina, cervix and two uterine horns. Two bilateral oval structures were seen at the proximal end of the uterine horns, the right measuring 2 x 1.7 cm (figure 2A), and the left 1.5 x 1 cm (figure 2B).

Microscopically, the structure on the right side of the animal represented a testicle, with numerous solid seminiferous tubules filled with immature Sertoli cells and germ cells, in which spermatozoids were not observed. Psammomatous calcifications were noted in the centre of the tubules. The

interstitium was abundant, with undifferentiated spindle cells. No mature Leydig cells were observed. The epididymis was present, surrounded by a tunica albuginea of dense irregular connective tissue (figure 3A).

The smaller structure on the left side represented an ovotestis, with secondary follicles, corpus luteum and corpus albicans. The stroma was abundant. The testicular component contained seminiferous tubules lined by sertoli cells. No spermatogenesis was seen in these tubules (figure 3B).

Uterus and uterine horns were of normal histological organisation.

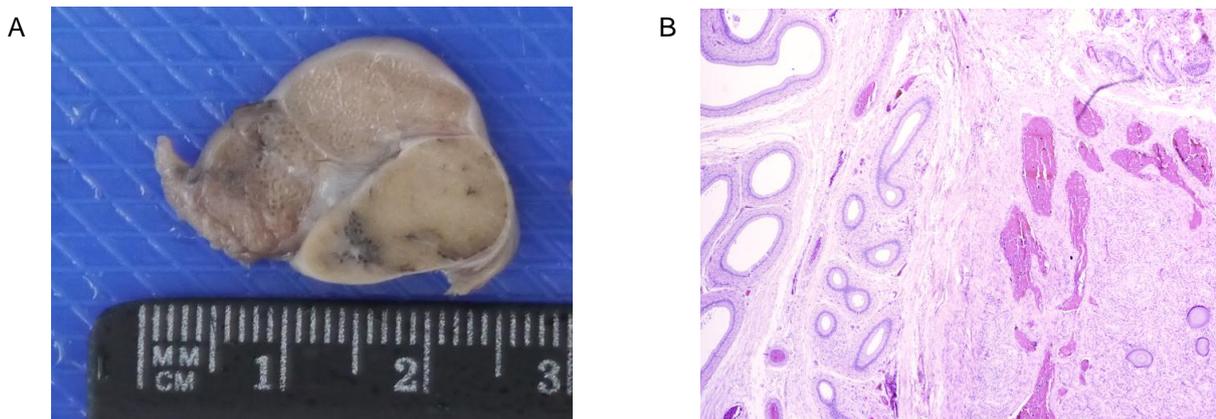


Fig. 2: Right "testicle-like" structure; A) Macroscopic image, cutting surface (Image: Pires I); B) Microscopic image (image: Prada J). Note testicular and epididymis histological components (H&E, 100x). (Both images: ©Veterinary Hospital of University of Trás-os-Montes e Alto Douro)

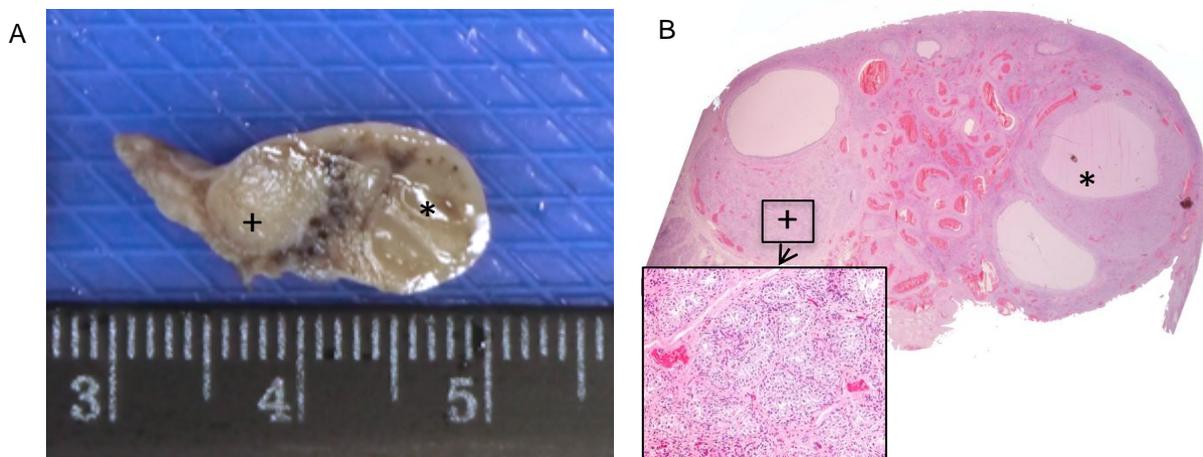


Fig. 3. Left "ovary-like structure" (ovotestis); A) Macroscopic image (cutting surface) with two different areas; B) Microscopic image. Note ovary components as secondary follicles and corpus luteum (), (H&E, 7,5x) and testicular component with seminiferous tubules lined by Sertoli cells (+), (H&E, 100x). (Photos: Pires I, Prada J / ©Veterinary Hospital of University of Trás-os-Montes e Alto Douro)*

Discussion

The existence of antlers and retained velvet is rare. In most cases it occurs with older females with a below average body condition and is probably secondary to hormonal changes related to previous

pregnancy (FLIS, 2009). By contrast, this case was of a young animal estimated to be 3 or 4 years old, in good bodily condition and showing no signs of recent pregnancy.

Moreover, this roe deer was not a female, but a true hermaphrodite. Examination of internal genitalia confirmed both female and male genital organs. The animal presented a uterus with two uterine horns and at its extremities of which were two structures: a testicle and an ovotestis, containing both ovarian and testicular histological structures. The presence of these structures seems to be the cause of the secondary sexual features, as well as the well-developed antlers.

The occurrence of hermaphroditism with the presence of an ovotestis is a rare phenomenon and refers to gonads containing both ovarian follicles and testicular tubular elements within them. Another case of true hermaphroditism in roe deer was described by PAJARES *et al.* (2009), but in contrast to the present case, ovaries and testicles were two independent anatomical structures. To the best of our knowledge, this is the first report of a true hermaphrodite with an ovotestis, in a roe deer.

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EYE PROBLEMS IN PINNIPEDS – FIRST RESULTS FROM GERMAN FACILITIES

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A survey, conducted by the authors, amongst Central European countries (Germany, Switzerland and Austria) reported on a prevalence of 36 % of ocular problems within the pinniped population in captivity (27 % within California sea lions (*Zalophus californianus*) (CSL), 48 % within European harbour seals (*Phoca vitulina*) (EHS)). A study has been initiated to verify and specify the reported issue. These are the first results taken from three exemplary facilities.

Within three German facilities (2 zoos, 1 circus) 88 % of the resident pinnipeds underwent an ophthalmic examination. On 16 California sea lions (seven males, nine females, age 3 - 23 years) and five European harbour seals (two males, three females, age 4 - 18 years) an examination with focal light source (all), photographic documentation (all), fluorescein staining (seven animals), slit lamp microscopy (four animals) and tonometry (one animal) was performed.

Fifty-seven percent of the examined pinnipeds had pathologic findings (56 % CSL, 60 % EHS). Twenty-nine percent of the pinnipeds showed acute symptoms (25 % CSL, 40 % EHS). Sixty-seven percent of the affected CSL displayed corneal involvement and 22 % lenticular changes. Thirty-three percent of the affected EHS displayed corneal involvement and 33 % lenticular changes.

Adequate medical training is essential for examining the pinniped eye. The results reveal a severe ocular health issue in Europe, comparable to data published from North America. Further research should be undertaken to study on potentially contributing environmental factors.

A RETROSPECTIVE REVIEW OF PATHOLOGICAL FINDINGS IN CEPHALOPODS SUBMITTED TO INTERNATIONAL ZOO VETERINARY GROUP PATHOLOGY BY SEA LIFE CENTRES

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This retrospective study catalogues *post mortem* histopathological findings in cephalopods submitted to a specialist diagnostic laboratory (IZVG Pathology) from Merlin Entertainments Group's Sea Life attractions during the period May 2003 to June 2014. Diagnoses were classified by pathological process and aetiology where possible. Submissions from 152 cephalopods were received, across 16 different species. The most frequent were common octopus (*Octopus vulgaris*, 25.0 %), giant Pacific octopus (*Enteroctopus dofleini*, 16.7 %), California two spot octopus (*Octopus bimaculoides*, 15.2 %) and chambered nautilus (*Nautilus pompilius pompilius*, 12.9 %). For octopus species across all years, the distribution of cases by pathological process was: no pathologic diagnosis (19.4 %), infectious and/or inflammatory (78.5 %), degenerative (3.2 %) and reproductive (2.2 %). The most significant transmissible agents in octopus were: coccidia (45.8 %), bodonid and/or ancistrocomid protozoa (13.5 %), bacteria (13.5 %), cestodes (8.3 %), nematodes (4.2 %) and Epitheliocystis-like agents (2.1%), and (probably commensal) dicyemid mesozoans were found in 41.7% of submissions. The prevalence and severity of coccidial infections differed between octopus species. For nautilus, no pathologic diagnosis was made in 18.7 % of cases, whilst 81.3 % had disseminated multi-organ inflammatory lesions. Little is known about the prevalence and significance of disease in aquarium cephalopods, and these results provide a starting point for future developments in husbandry and disease investigation.

CORTISOL DETECTION IN NEW MATRIXES FROM SEVERAL MARINE MAMMALS: VIBRISSAE, BALEEN PLATES AND SHED SKIN

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The quantification of glucocorticoids, whose production results from the activation of the hypothalamic-pituitary-adrenal axis, has been widely used as a tool to assess stress responses of animals. Cortisol, the main glucocorticoid in most mammals, has been mainly detected in serum, urine, saliva and faeces. Hair has recently been developed as a new matrix to detect long-term retrospective cortisol levels. We hypothesised that hormone accumulation could also be possible in vibrissae, baleen plates or shed skin due to similarities with hair in growth and accumulation mechanisms. Different samples from living captive animals, including vibrissae from a Patagonian sea lion (*Otaria flavescens*) and from two walrus (*Odobenus rosmarus*), shed skin from a beluga whale (*Delphinapterus leucas*), and baleen plates from a dead stranded fin whale (*Balaenoptera physalus*) were analysed. Cortisol was detected in all the matrixes evaluated, using an enzyme immunoassay (EIA) and following a modified hair cortisol extraction protocol. The EIA kit used was initially developed for plasma cortisol assessment but later also validated to detect cortisol in hair samples of some domestic and wild species. Five vibrissae from a sea lion were analysed: two long vibrissae were divided in proximal and distal segments according to anatomical differences and three small vibrissae from the same animal were combined prior to analysis. High differences in cortisol concentrations were detected between proximal parts, distal parts and the group of small vibrissae (11.37 ± 0.04 pg/mg, 0.92 ± 0.08 pg/mg and 4.64 pg/mg, respectively). Differences between individuals were also detected in walrus vibrissae from two animals (1.93 pg/mg vs. 0.72 pg/mg). Cortisol concentration in the sample of dried shed skin from a beluga whale was 0.85 pg/mg. Samples from the fin whale also showed differences in cortisol levels between compacted baleen plates and non-compacted bristles (1.24 pg/mg versus 6.23 pg/mg, respectively). In conclusion, this preliminary study indicates that cortisol in vibrissae, baleen plates and shed skin was detected by the EIA. A full validation procedure is however necessary to assess if this method can be used reliably in the context of long-term retrospective endocrinology and wildlife welfare assessment.

EXPLORATORY COELIOTOMY AND REMOVAL OF AN ENTEROLITH FROM A SAND TIGER SHARK (*CARCHARIAS TAURUS*)

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Over a period of 18 months sporadic feeding was observed in a previously healthy adult female sand tiger shark housed at the SeaLife London Aquarium. This led to severe weight loss and revealed a large mobile mass in the abdomen of this 2.7 m shark. Due to the condition of the shark and the failure of separate treatments with enrofloxacin (Baytril™ Bayer PLC, Newbury West Berkshire, UK), carprofen (Rimadyl™ Zoetis UK Ltd, London, UK) and nandrolone (Laurabolin™.MSD Animal Health, Milton Keynes, UK) an exploratory coeliotomy was performed.

Surgery was performed under anaesthesia with MS222, (Tricaine Pharmaq™, Pharmaq, Fordingbridge, Hampshire, SP6 1PA, UK) the mass was found in the spiral valve where it appeared to be partially obstructing the intestine, it was approximately 10 cm in diameter and spherical in shape. Following removal of the enterolith, the tissues were sutured closed and the shark was returned to the water but died seven hours post surgery.

The mass was identified as an enterolith; these are formed from concentric layers of mineral deposits around a central nidus. X-ray diffraction analysis showed that it consisted of calcium hydroxyapatite (calcium phosphate carbonate) the main component of fish scales and skeletons. The nidus is usually an indigestible item; in this instance an octopus beak. Octopuses are used to feed this exhibit, though it is possible the beak was consumed in the wild.

The exact reason the enterolith formed is unclear but changes in the pH of the intestine and a diet high in minerals can contribute to their formation following the ingestion and intestinal impaction of any indigestible object capable of acting as a nidus.

Post mortem examination and histopathology did not reveal the cause of death. The appearance of the liver was consistent with the history of weight loss, implying significant loss of reserves of body fat, probably affecting the shark's ability to cope with capture, anaesthesia and surgery. Sections of spiral valve / large intestine showed no major histological changes to correspond with the impacted enterolith.

CAESAREAN SECTION ON A BLUE SPOTTED RIBBONTAIL RAY (*TAENIURA LYMMA*) USING A DORSAL APPROACH

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Blue spotted ribbontail ray (*Taeniura lymma*) have been bred in captivity, but this was a first for the National Marine Aquarium, UK. We had also observed and filmed copulation. This led to close monitoring of the pregnancy. All had seemed to go well with an ultrasound examination confirming the live pup active in the uterus.

A sudden loss of fluid and subsequent non-emergence of the pup, at a time where normal birth could be expected, lead to the concern it had died in the uterus and would lead to complications.

The ray was examined and when it was clear the pup could not be delivered manually, the decision was taken to carry out an emergency caesarean section under MS222 (Tricaine Pharmaq™, Pharmaq, Fordingbridge, Hampshire, SP6 1PA, UK) anaesthesia. Ultrasound examination suggested that the dorsal approach was possible and avoided the problems associated with the position and size of the liver when approaching through the more traditional ventral midline.

The 285 g dead pup was removed through an 8 cm paramedian incision 3 cm from the midline. The uterus and coelom were closed in a standard way using 4/0 Polydioxanone (PDS™, Ethicon, Johnson & Johnson Medical Ltd, Somerville, New Jersey, USA). PDS and Polyglactin 910 (Vicryl™, Ethicon, Johnson & Johnson Medical Ltd, Somerville, New Jersey, USA) were used alternately for the simple interrupted sutures in the skin to try to reduce the possibility of wound breakdown.

The ray recovered well with no signs of nerve deficit from the surgery.

VASCULAR HEART DEFORMATION IN A HARBOUR PORPOISE (*PHOCOENA PHOCOENA*)

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A congenital heart defect in harbour porpoises (*Phocoena phocoena*) has been described (JEPSON and ROBINSON, 1995) only once. The present case-report describes a severe heart malformation in a juvenile female harbour porpoise. The animal was found dead on October 17th 2013 at the coast of the island Amrum in the German North Sea, weighing 15.6 kg, measuring 91 cm in body length and in good body condition. *Post mortem* examination revealed a large, oval-shaped and bluntly coned heart with cardiac hypertrophy and an absolute weight of 398 g compared to an average of 136.5 g of other necropsied juvenile porpoises with a similar body weight ($n = 15$). The relative heart weight was 2.55 % compared to the reference value of 0.52 %, measured by ROWLATT and GASKIN (1975). The heart showed a severe right ventricular dilatation and an abnormal wide vessel originating from the aorta, which continued around the coronary sulcus of the left ventricle and entered in the right ventricle. This abnormal wide vessel was identified as *Arteria coronaria dextra*. The left ventricle showed a hyperplasia of the *Trabeculae septomarginales*. These defects generated a severe blood shortcut, which may have compromised the oxygen supply in the periphery, but no significant changes indicating a shortage in oxygen supply was found in other organs. The congestion and oedema of the lungs can be linked to this heart defect, but might also be caused by agony around the time of death. At histology, cardiomyopathy and hypertrophy of both ventricles were observed.

Concluding the findings, the young harbour porpoise had a congenital malformation of a cardiac blood vessel resulting in cardiomegaly with dilatation of both ventricles and myocardial hypertrophy. There are no convincing changes of decompensation (e.g. serous effusions) or oxygen shortage (e.g. degeneration of centrilobular hepatocytes) and the cause of the spontaneous death of the animal remains obscure, but the severe heart defect may have contributed to the death of this animal. Due to the good body condition status, a sudden death may be suspected.

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PRELIMINARY INVESTIGATION OF A POTENTIALLY ECONOMICAL IODINE SUPPLY FOR LARGE PUBLIC MARINE AQUARIUMS

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In aquariums around the world countless Elasmobranch specimens show the symptoms of goiter. Goiter is caused by a swelling of the thyroid gland that can enlarge to more than 300 times its normal size. If left untreated, it may result in starvation and finally death. The development of goiter in elasmobranchs is related to insufficient iodine supplementation.

The causes of iodine deficiency in public aquaria are the low natural iodine content in the forage, the low iodine content of the salt mixture and the de-ionisation which occurs during water purification.

Replacement of iodine is carried out according to the present practice by: (i) enrichment of the water with various additives, or (ii) charging such vitamins in the forage which contain iodine.

The first method is very costly, and when the second one is used, the absorption of iodine is much smaller when compared to direct absorption through the gills of the animal.

In most cases, maintaining iodide concentrations close to a natural level (i.e. 0.06 mg/L) and lowering the nitrate concentration (< 10 mg/L) will reduce the prevalence of goiter. For water supplementation with iodine, large quantities of additives produced by commercial companies, have to be used in the case of large public aquarium tanks, which means high expenses for the institutions. The addition of iodine tablets to the feed is not always possible because some individuals refuse feed that has been treated with the supplement.

The authors at Nyíregyháza Zoo (Sosto Zoo) have worked out an alternative method for iodine dosing during the treatment of a whitetip reef shark (*Triaenodon obesus*) and a nurse shark (*Ginglymostoma cirratum*) which has advantages in comparison to the previously described techniques, such as cost effectiveness, easy workability and reducing the risk of overdose. The charging of iodine is achievable through a reaction chamber, which contains iodine crystals, via air flowing; the iodine saturation of the air which flows into the water is determined by the strength of the air flow and the amount of crystals of iodine. Measuring the loss of the iodine crystals in the reaction chamber and measuring the iodine-concentration of the water provide opportunities to control the operation of the system at two points.

Two cases of a successfully completed medical treatment performed by the authors via the above described system have broadened the horizons towards the development of this method.

TUTORIAL IN MODELLING BODY MASS DYNAMICS OF YOUNG WILD MAMMALS: APPLICATION TO TWO EXAMPLES

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It is interesting to focus on the body mass dynamics of young wild mammals in several situations, such as the comparison of the growth patterns in different populations of the same species. Modelling the body mass dynamics also allows testing the associations between individual characteristics and growth parameters given by the model, such as the average daily weight gain.

The aim of this presentation is to provide a step by step approach to build and use models characterising the body mass dynamics in young wild mammals.

The first part of this tutorial presents the theory and the construction steps to model the body mass dynamics of 49 (29 males and 20 females) young hand-reared clouded leopards (*Neofelis nebulosa*) during their first 90 days of life. The cubs from three different conservation centres (18 litters from Khao Kheow Open Zoo in Thailand, three litters from the Center for Species Survival of the Smithsonian Conservation Biology Institute in Virginia and three litters from Point Defiance Zoo and Aquarium in Washington State) were fed the same formula mixture after a similar three steps weaning protocol set by the Smithsonian Institution. The approach to build a database and find a model fitting with the collected weights is explained, while referring to this practical example. Finding a growth pattern is valuable as an aid in managing this rare species, and can help identify early onset of medical issues.

The second part of this tutorial comments the analysis of the body mass dynamics of 645 gray mouse lemurs (*Microcebus murinus*), raised by their mother with their potential brothers and sisters in the colony of the Muséum National d'Histoire Naturelle in Brunoy (France), during their 100 first days of life. The non-independence of the data on several levels and the choice of a linear mixed-effect model with one change in slope instead of a more complicated nonlinear model (Gompertz model) are discussed. The unadjusted and adjusted associations between the body mass dynamics and some individual characteristics of the young animals (such as their sex, their mother's parity and the number of other offsprings in their litter) are analysed and interpreted. The modelling of early growth in these little primates genetically very close to humans could be useful in wider studies about the long-term costs of growth on longevity and reproduction performance.

This presentation shows that using mixed-effect models allows to take into account the non-independence of the data and to choose which type of regression models (linear or non-linear, with or without change(s) in slope(s)) is the best to describe the body mass dynamics of young wild mammals in their first days of life, in most of the situations.

PORENCEPHALY IN ADULT PHOCIDS

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Cavities in the brain may result e.g. from dilatations of liquor-containing spaces (hydrocephalus), the loss of neuroparenchyma with development of cystic lesions (porencephaly) or abscess formation. Hydrocephalus is reported, mainly as congenital anomaly, in young seals, whereas cases in adult phocids have not been described yet.

A weakened, wild, adult, male grey seal (*Halichoerus grypus*) had been shot at the German coast of the Baltic Sea in August 2014. MRI and pathological examination showed a bilateral, asymmetrical porencephaly and a symmetrical internal hydrocephalus with dilatation of both lateral ventricles and volume expansion of the brain. The neuroparenchyma adjacent to the porencephalic cavities displayed gliosis and occasionally a mild perivascular lympho-histiocytic infiltration. Additionally, *Sc. dysgalactiae* subsp. *equisimilis* were isolated from the lung, spleen, kidney, intestine, testicles and skin in high microbial counts.

A male harbour seal (*Phoca vitulina*), born in captivity, showed atypical behaviour of a newborn seal. It died at the age of 12 years spontaneously in September 2014 due to cardiovascular failure caused by a severe uraemia associated with nephrolithiasis. The necropsy revealed a focal loss of neuroparenchyma in the left temporal cortex consistent with a porencephaly. The cavity communicated with the lateral ventricle. In the adjacent neuroparenchyma mild gliosis, vacuolisation and mineralisations were present. The focal loss of neuroparenchyma with cavity formation is consistent with porencephaly.

The pathogenesis in both cases remains undetermined. Mild inflammation of the brain adjacent to the porencephalic cavities in the grey seal may indicate a post-inflammatory lesion. The internal hydrocephalus in this individual has most likely contributed to the volume expansion of the brain. Whether the internal hydrocephalus represents a separate disease process or a lesion associated with porencephaly cannot be determined with certainty. With respect to the history in the harbour seal a congenital porencephalic lesion due to an unknown intrauterine damage cannot be excluded. However, in both cases other causes (e. g. chemicals, virus, and trauma) are possible.

VETERINARY SERVICE FOR “SABRE-TOOTHED SAUSAGES” – EXPERIENCES WITH THE NAKED MOLE-RAT (*HETEROCEPHALUS GLABER*) AT ZOO DRESDEN

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Summary

Veterinary care for naked mole-rats (*Heterocephalus glaber*) poses special challenges due to the species' unique features. The small rodent, that has been an object of great interest for many scientists, has been bred at Zoo Dresden for several years with increasing success. An overview of some of the clinical procedures, pathological conditions, and drugs commonly used at the authors' facility is provided.

Introduction

The naked mole-rat is a rodent species endemic to the dry regions of Somalia, Kenya, Djibouti and Ethiopia (MAREE and FAULKES, 2008). It is known for its social and strictly subterranean lifestyle (LACEY and SHERMAN, 1991). Family groups usually consist of 75 to 80 individuals, but groups from 10 to 290 animals have been described. Within a family group only one alpha-female and one to three males are reproducing, while the other members directly or indirectly assist in rearing the offspring, and build and defend the tunnel system (BRETT, 1991). Naked mole-rats can live more than 28.3 years (BUFFENSTEIN, 2008). They have fascinated scientists for a number of reasons, including their social structure, longevity, cancer resistance, nociception, vitamin D and calcium metabolism and tolerance to low oxygen levels and temperatures (FAULKES and ABBOTT, 1991; PITCHER and BUFFENSTEIN, 1995; BUFFENSTEIN, 2008; PARK *et al.*, 2008; LARSON and PARK, 2009).

Zoo Dresden has been keeping naked mole-rats since 1995, when the first group was brought in from Columbus Zoo (Ohio, USA). Since the arrival of a second group in 2003, efforts in captive breeding have grown very successful. In 2014, 187 offspring were born, of which 117 have been transferred to various zoological institutions. To date, Zoo Dresden is keeping about 70 individuals in multiple groups. Zoo Osnabrück and Tierpark Berlin are the only other German zoos keeping naked mole-rats, while 15 more institutions are registered within the EAZA.

Materials and methods

The following passages provide a short overview of some of the diagnostic procedures and medications but also pathological conditions diagnosed in naked mole-rats kept at Zoo Dresden. Clinical procedures and medications are often limited by the small size and to a certain point by the high sociality of the naked mole-rat, which usually does not allow for a long separation from the group. Health evaluation is highly dependent on the observation of behavioural aspects like social interactions, activity level and observed food uptake. Additionally, individuals of different behavioural groups and age classes (older and younger alpha-females, reproductive males, new-borns, youngsters and adults, etc.) may differ markedly in what is considered “normal” or “abnormal”

behaviour and appearance. The remaining animals of a group should be monitored for any abnormalities that might indicate a general problem, which makes an experienced keeper/observer an essential prerequisite. Clinical examination of the individual includes a check for body condition, precise and repeated weight assessment, hydration status (skin turgor and moisture), examination of the body surface and orifices, abdominal palpation, as well as jaw position and tooth wear. Faecal pellets are frequently produced during a clinical procedure by the non-anaesthetised animal and can be examined using standard methods. Abdominal organs may be partially visible through the translucent abdominal wall.

Due to their lively and agile manner as well as their painful bite, most procedures are performed under general anaesthesia, which is safely obtained with 5 % isoflurane (Isofluran-CP[®]; CP-Pharma Handelsgesellschaft mbH, Burgdorf, Germany) in oxygen in an induction chamber. During induction, the animals commonly exhibit signs of discomfort, like increased mobility and rubbing of the nose with the forefeet. Anaesthesia is maintained with 1 – 3 % isoflurane in oxygen via facemask, while short interruptions of anaesthetic gas delivery (e.g. for radiography or dental examination) do not result in a quick reduction of anaesthetic depth. Care has to be taken to avoid hypothermia, and appropriate measures like heated pads and pre-heated subcutaneous fluids should be prepared ahead of the procedure. In the authors' experience, recovery from isoflurane anaesthesia is often prolonged and may take up to 20 min.

Small amounts of blood for the assessment of basic haematologic parameters can theoretically be obtained by clipping the tip of the tail in the anaesthetised animal. Given the invasive character and bad repeatability of the procedure as well as the small size of the animals, this is usually not considered. Reference values of serum chemistry and haematologic parameters for the species are lacking.

Computed radiographic imaging using high-resolution dental films may aid in the diagnosis of dental, skeletal and gastrointestinal conditions. Intestinal passage with contrast media (BaSO₄) has been performed in one occasion. Ultrasound examination is not commonly done at the authors' facility, but detection of pregnancy or abdominal fluid accumulation and ultrasound-guided abdominocentesis have been performed. Oral and pharyngeal examination can be conducted with a rigid endoscope.

Some of the pathological conditions which affected the naked mole-rat population at Zoo Dresden are severe infestation with the tropical rat mite (*Ornithonyssus bacoti*), bite wounds inflicted by conspecifics and skin abscesses, suspected transient radial nerve paralysis after a fight, mandibular luxation, overgrown incisors, intestinal obstruction ileus, catarrhal enteritis, gastric ulcer, intestinal infestation with flagellates (*Trichomonas* sp., *Hexamita*) and nephropathy/nephrocalcinosis.

Intestinal pathogens found in faecal samples of sick animals or during necropsy of animals with enteritis included *Klebsiella pneumoniae*, haemolytic *Escherichia coli*, *Candida tropicalis* and *Candida albicans*.

The use of drugs in naked mole-rats is often restricted by the small size and lack of knowledge on species-specific tolerance and dosages. A number of drugs have been used at the author's institution. In our experience, subcutaneous injection of fluids and drugs seems to be particularly uncomfortable and is usually performed under general anaesthesia. The following list shows medications being used by the authors:

- topical selamectin (Stronghold[®] Spot on for kittens < 2.5 kg, 1 drop/animal; Zoetis Deutschland GmbH, Berlin, Germany) in case of mite infestation,
- oral trimethoprim/sulfadoxin (Borgal[®] 24 %; Virbac Tierarzneimittel GmbH, Bad Oldesloe, Germany), and
- enrofloxacin (Baytril[®] 0.5 % oral solution for pigs; Baytril[®] 2.5 %; Bayer Vital GmbH, Leverkusen, Germany), in case of bacterial infections,

- oral meloxicam (Metacam® 0.5 mg/ml suspension for cats; Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany),
- oral simethicone, oral lactulose, probiotics in cases of bloat, obstipation or diarrhoea,
- oral B-vitamins in cases of neurologic disorders or for general support in weak animals,
- warmed SC fluids (NaCl 0.9 %, Ringer's-Solution, Dextrose 5 %) in dehydrated and anorectic animals.

Oral solutions and suspensions can be diluted to achieve a suitable concentration. Dosages and application are used in accordance with other small rodent species, as species-specific dosages are missing.

Results and discussion

Despite the animal's small size, a diagnosis can often be made with the right effort and equipment. For early recognition of abnormalities and medical conditions, but also successful management, knowledge about species- and group-specific behaviours is essential. Naked mole-rat medicine is still in its infancy, and an exchange of experiences regarding diagnostic and clinical procedures as well as proper medications is being encouraged by the authors.

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THE USE OF A NEW AUTOLOGOUS REGENERATIVE BLOOD-DERIVED PRODUCT FOR WOUND AND LESION HEALING IN ELEPHANTS

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During an outbreak of a cowpox-infection the total group (13) of elephants (*Elephas maximus* and *Loxodonta africana*) in Cologne Zoo developed mild to severe horn lesions in their hooves. Pox vesicles on the feet are a severe complication of this infection. Those vesicles undermine the horn, the sole and the skin above the horn. This could lead to the complete loss of the horn/tissue unit and in severe cases the animal's death. With exception of one animal all were affected by hoof lesions of variable severity. All lesions were surgically treated. Local treatment was done by bathing the feet daily in solutions with antiseptics and the local application of wound healing ointments and creams to control the infections and stimulate wound healing. Three animals suffered from multiple erosions and showed signs of pain and received a supporting treatment with the oral antiphlogistic and anti-inflammatory drug Phenylbutazon (Butazolidin[®], MSD, Animal Health, Chicago, USA). Despite these treatments open wounds persisted, probably due to a non-reliable application. Wild animals lack cooperative behaviour, they have no possibility for containment avoiding licking and biting, and daily narcosis is not an option. A new autologous regenerative product optimised for 'in vivo' wound healing, containing platelet rich plasma and major bioactive growth factors was used (PLT^{fix}[®], Fat-Stem Laboratories, Aalst, Belgium). When PLT^{fix}[®] is applied, the damaged tissue should stimulate the release of growth factors from the platelet's granules, which access and augment the healing process and promote the removal of inactive, dying or necrotic tissue. Oral reports and ultrasonography of satisfying results from domestic equine medicine led to close contacts and skill enhancement for the use of PLT^{fix}[®] in these elephants. The three elephants with most severe lesions and signs of lameness were tested. Anti-coagulated blood (100 ml) was retrieved from the hind legs and sent to the Fat Stem laboratory. PLT^{fix}[®] was obtained by density gradient centrifugation and an 'in house' concentration technique. Platelet concentration is 5x higher and the white blood cell count is 2.5x lower compared to whole blood. Within 72 h after blood retrieval the PLT^{fix}[®] was sent back (frozen) in doses of 1.5 ml to the zoo for therapeutic application. In domestic horses the PLT^{fix}[®] is injected locally around the margins of the lesions into the healthy parts. However, the elephants did not tolerate this local injection and therefore PLT^{fix}[®] doses were mixed with 10 % DMSO (Wak-Chemie Medical GmbH, Steinbach, Germany), acting as a carrier additive helping the active ingredients being transported through the skin tissue. One dose was applied one time to a single lesion by moisturisation. No side effects were observed. The first five days no changes were observed. After one week a fast growth of a firm regenerative granulation tissue was seen. The lesion was covered and regarded as healed. After two weeks the normal horn started to grow from the top and the sides. By that time lameness got resolved, indicating a decrease in inflammation and pain. Within a month the affected area was difficult to be told apart from the non-affected area. This was seen in all three animals which received the PLT^{fix}[®], but not in the other zoo elephants. PLT^{fix}[®] proofed to stimulate wound healing, horn production and limits the spread of the ulcerative skin in treatment of infection-associated lesions in the elephant feet. It is of additive value as it gives the option for a simple one-time application and rapid regeneration. PLT^{fix}[®] should become a standard for the daily hoof trimming in elephants. It is intended to use this method in other wound healing-associated disease as well as in other animals. The Köln Zoo and Fat stem are working on a gel application for the product.

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