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(EAZWV)

European College of Zoological Medicine
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PROCEEDINGS OF THE ZOO AND WILDLIFE HEALTH CONFERENCE 2019

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30 YEARS OF EXPERIENCE IN WILDLIFE RESEARCH

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Based on three decades of clinical and experimental work with free-ranging mammals, the brown bear (*Ursus arctos*) is used as an example to show the development of immobilization techniques, from the pioneer days with etorphine and practically no monitoring to the current best-practice (*lege artis*) protocols for field anesthesia and surgery. Additionally, highlights from research topics such as ecophysiology, bio-logging, translational medicine, wound ballistics and environmental lead exposure are presented.

SCREENING FOR LEAD IN SCANDINAVIAN BROWN BEARS (*URSUS ARCTOS*)

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Lead (Pb) exposure is a health risk associated with adverse health effects in humans and wildlife. Blood lead levels (BLL) of sentinel wildlife species can be used to monitor environmental lead exposure and ecosystem health. Blood lead analysers, such as the LeadCare® systems, are validated for use in humans, assessed for use in some avian species and cattle, and are increasingly being used on wildlife to monitor lead exposure.

The LeadCare® analysers use anodic stripping voltammetry (ASV). Species-specific conversion equations have been proposed to estimate the concentrations found with gold standard measuring methods, such as inductively coupled plasma mass spectrometry (ICP-MS) because the ASV method has been shown to underestimate BLL in some species.

In this study we assessed the LeadCare® Plus (LCP) for use on Scandinavian Brown bears (*Ursus arctos*) by comparing the BLLs with ICP-MS results on both fresh (N=54, 2018) and frozen (N=70, 2010-2017) samples, and screened the population for exposure.

We found a high correlation between the two methods and identified a Bland-Altman analysed bias of 16.3–22.5% overestimation of the LCP. Based on linear regression, we provide conversion equations to estimate ICP-MS blood lead levels based on the LCP results within the observed range of this study (38.2–174.0 µg/L). We found a mean BLL of 90.23 µg/L and high correlation between the levels of mothers and their dependent offspring. The European Food Safety Authority level of concern for neurotoxicity in humans is 12 µg/L. Our findings indicate high lead exposure in Scandinavian brown bears.

VETERINARY FOLLOW-UP OF A LOCAL REINTRODUCTION PROGRAM (*EMYS ORBICULARIS*): FROM THE EGG TO THE WILD!

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In the context of the sixth crisis of biodiversity, reintroductions are considered a major forward-looking strategy for sustaining future wildlife. Reintroductions consist of releasing into the wild individuals born and grown in captivity, with two major issues: ensuring, in artificial conditions, survival and health of individuals from birth to release age and guaranteeing an appropriate transition to the natural environment and beyond. Veterinary follow-up of such conservation projects in their entirety is of utmost importance.

In Alsace (NE France) where the species got extinct during the 19th century, more than 280 European pond turtles (*Emys orbicularis*) have been reintroduced since 2013. The conservation program is part of the National Action Plan. It was assisted by a zoo veterinarian throughout its duration. Producing young turtles from eggs collected in a dedicated captive enclosure implied breeders monitoring and management, incubation setup, animal husbandry, then appropriate nutrition and regular health monitoring. Major improvements in captive setups, diet and veterinary care strongly enhanced both hatching and early survival rates and growth. Before release, quarantines were used to prevent sanitary risks for the native fauna on the releasing site. In the wild, monitoring of somatic growth, diet and dispersion of released individuals was carried out to evaluate the success of the reintroduction. Today, after-release survival is 86%.

Additionally, invasive species (namely red sliders *Trachemys sp.*) were removed from the release site field to enhance the program success. Specimens were brought to the zoo, sterilized and presented in a pond providing visitors' information to prevent further private releasing.

THE OTTER WAY OF WILDLIFE MANAGEMENT – OTTER-FRIENDLY EXITS FROM FISH FYKES SUCCESSFULLY DEVELOPED IN COLLABORATION WITH STAKEHOLDERS

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The European otter (*Lutra lutra*) is globally listed as being near threatened on the *IUCN Red List of Threatened Species*. In Germany, road traffic is the principal cause of death. Nevertheless, fish traps represent another imminent danger. If otters swim into the cod-end of a fish fyke to prey on the trapped fish, they can no longer free themselves and drown. Currently accepted otter protection measures are so-called otter guards or stop grids. These have the disadvantage that they collect debris and that catches of high-backed species significantly decline due to the constricted entrance. To proactively prevent future fatalities while still doing inland fisheries justice, this cooperative study between independent researchers, otter conservationists and fisheries representatives successfully developed two exits that allow otters to leave the fyke while keeping the catch inside. One exit comprises two flexible wire ropes held together by a loose latex wrap that rips once an otter tries to escape. The other consists of two fixed metal brackets connected by a spring, which open under pressure. Both exits are fitted in the cranial roof of the cod-end. By pushing their muzzle into the ensuing opening when searching for an escape route, the exits give way and the otter can easily leave the trap. Additional tests regarding fish retention revealed that different fish species of commercial relevance could not open the exits. Thus, both variants are respectable wildlife conservation methods that should be implemented in stagnant water fyke fishing practices as supplemental otter protection measures.

FROM THE “PUSZTA” TO THE STEPPE – LARGE SCALE PRZEWALSKI'S HORSE (*EQUUS FERUS PRZEWALSKII*) TRANSPORT FROM THE HORTOBÁGY NATIONAL PARK TO RUSSIA

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The Przewalski's horse (*Equus ferus przewalskii*) is a flagship species for many zoological institutions as active reintroduction takes place in Mongolia and other Asian locations. The Hortobágy National Park, Hungary hosts the biggest population under natural conditions in a so-called semi-reserve. In 2016 and 2017 large scale transports were completed, where 14 and 16 horses respectively were sent via air and road transport to a new reserve in Orenburg, Russia. This complex procedure included the selection of individual horses and extra animals to provide the travelling 30 animals; most of them were darted from a large distances due to the special conditions of the place of origin. All horses were anaesthetised with a detomidine, butorphanol, etorphine and acepromazine combination twice, once for marking, disease screening and transport to the field quarantine station and the second time on the day of the transport. The general dosage was 10 mg TD detomidine, 10 mg TD butorphanol, 2,45 mg TD etorphine and 10 mg TD acepromazine (the latter two compounded). Antidotes were given after the short procedures and the horses were calm and relaxed during the entire journey. This method provided a safe and effective handling of the animals (WALZER, 2014). The testing for a number of contagious diseases, the preventative measures taken against the possible complications occurring during a long transport and the carefully designed crates resulted in zero mortality transports, which could serve as a good example for similar activities in the future.

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NON-SURGICAL ARTIFICIAL INSEMINATION IN AFRICAN LIONESSES (*PANTHERA LEO*) AFTER OVULATION INDUCTION WITH A GNRH ANALOGUE

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Assisted reproduction techniques (ART) have incredible potential in wildlife conservation breeding. However, overall ART success in felids remains <25%. The aim of this project was to develop a non-surgical artificial insemination (AI) protocol for African lions using an exogenous GnRH analogue (20 µg buserelin-acetate IM, Receptal®, MSD, Intervet, South Africa) to induce ovulation in females presenting natural oestrus. This methodology was preferred over the previously described timed-AI approaches for non-domestic felids (laparoscopic insemination after ovulation induction with gonadotropins), to avoid the potential risks associated with repetitive use of eCG and hCG, and surgery.

Four protocols, differing in the time lapse between GnRH injection and AI, on days 4, 5, or 6 after the onset of natural oestrus (determined by daily behavioural observation and vaginal cytology), were tested in five trained lionesses, in 14 trials. Fresh semen was collected from 8 males by urethral catheterization and electro-ejaculation during full anaesthesia. After transrectal ultrasound examination of the reproductive tract, the anaesthetized female was inseminated either intra-vaginal or trans-cervical by a commercial dog urinary catheter (2.0 x 500mm, Buster®, Krusse, South Africa).

Exogenous GnRH administration successfully induced ovulation in all females. All lionesses entered either a non-pregnant luteal phase (pseudopregnancy) of 59.6 ± 0.95 days ($n=10$) or a pregnant luteal phase of 111.67 ± 0.33 days ($n=3$). However, the timespan between GnRH injection and end of behavioural and/or cytological oestrus differed widely (72.86 ± 11.28 hours; range: 0-120). The final pregnancy success rate was 33.3%. This method has potential to be applied to other large felid species.

AVIAN EGG INCUBATION: BETWEEN SCIENCE AND ART

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Abstract

Unluckily, there is not much scientific literature about the incubation of non-domestic bird eggs, thus the reading list is necessarily short. However, both authors are bird breeders, or are consulting several bird breeding facilities, including some among the largest bird collections in the world, so the information given in this paper is widely taken from non-veterinary books, personal experience, personal communication with breeders and bird keepers.

Natural incubation

Unlike other zoologic groups, birds do not abandon their eggs but actively create around the egg the correct conditions for the growth of the embryo. This process is defined as incubation and corresponds to the gestation of mammals. The embryos are exposed to additional risks outside the body of the female, but, apart from the obvious advantage of not burdening the female with pregnancy, makes possible the cooperation of both sexes in the process.

Incubation, with the exception of very few bird species, is conducted through an adult individual who sits on the eggs, heating and moving them. This action is defined as brooding. The brooding duty, depending on the species, is a responsibility of only the cock (e.g., most Ratites), only the hen (Galliformes, Anseriformes, several songbirds), or both members of a pair (almost all Psittaciformes and Columbiformes).

Artificial incubation

There are several reasons to artificially incubate (AI) avian eggs. Among the most common reasons to incubate artificially avian eggs, we may list:

- Need to increase the production of eggs/chicks (commercial farm; breeding project of a threatened species).
- Very high biological value of a given species
- Very high financial value of a given species
- Ability/inability of a given pair to rear chicks successfully (e.g., they break the eggs, do not take care of chicks)
- Experience of a pair: sometimes a young pair in a valuable species is not yet able to incubate and rear chicks; this may lead the owner to collect the first clutch to guarantee at least some chicks.

Good management of AI and hand rearing techniques may guarantee an increment in the number of chicks, both for the pet bird market and restocking the collection. However, without long-term planning,

the breeding stock could become easily exploited, with a shorter production life and long-term stress that will expose the breeding birds to diseases. Importantly, if the breeder wants to use AI well and hopes to achieve good results, this involves a significant commitment at several levels:

- Financial (incubators, furniture, setting up one or more special rooms).
- Time for record keeping.
- Time for hand rearing (artificially incubated chicks from altricial species must be hand reared).

A good plan for AI must be designed to meet the needs of the owner/curator. But there are several other factors to take into account:

- What is the main role of the owner/curator?
- What kind of experience with artificial incubation and hand rearing does he/she have?
- How much time does he/she have to spend in both activities?
- Number of breeding pairs in the facilities?
- Species in stock?
- Are the breeding birds experienced in rearing chicks?
- Is there any specific disease that might lead to different breeding techniques in the breeding facility? (e.g., diseases that are not transmitted vertically and make AI a good option).

Finally, although there is not a “gold standard” for a good incubation plan, AI has specific rules, and if the breeder does not follow the rules, results could be disappointing.

Egg formation and structure

Knowledge of the structure of an egg is mandatory for a successful incubation. Further, the egg must contain all the needed nutrients to support the good growth of the embryo. Adequate nutrition of the breeding birds, but especially of the female, is of primary importance. The egg is composed of five fundamental parts:

1. Egg yolk
2. Egg white
3. Chalazae
4. Shell membrane
5. Eggshell

Prerequisites for artificial incubation of eggs

The most important prerequisite for eggs incubation is fertility. This can be assessed by observing the blood vessels within the egg, which can be seen very early after egg laying. Also, a good egg is not misshapen, has a smooth and even surface, and is not cracked.

It is better not to incubate broken or cracked eggs; however, if one decides to do so, the egg must be repaired. For the purpose, glue or candle wax can be used, but they tend to seal several eggshell pores around the crack and may limit egg respiration. For these reasons, altered eggs should be considered potentially infected; thus, it is better to incubate them in a separate incubator.

Record keeping

Developing a good record keeping method will help the breeder to evaluate the previous breeding history of the birds and make the right decisions about incubation procedures. To keep records well, some data will be written on the eggshell, and other information will be recorded somewhere else. The best tool for writing on the eggshell is a blunt, soft pencil.

Information that should be recorded on the eggshell includes:

- Date of laying
- Species
- ID N° of the pair, or female, or aviary.

Somewhere else (generally a spreadsheet on the computer) we shall record:

- Egg weight (and size, if possible)
- Any abnormality (e.g., cracks, strange shape)
- Date when incubation actually starts.

Storing eggs

It is better to allow the eggs to “rest” for a few days (2-5) before starting incubation, as this seems to increase hatchability of fertile eggs. Recently laid eggs are harvested from the nest and allowed to cool down a bit.

Then the eggs are placed horizontally in a clean container and laid on a very soft substrate (i.e., millet seeds, very dry sand, specific trays for egg storing). Eggs can be stored at 13° to 16° C for up to 5 days. It is still debated whether it is better to leave them as they are or to turn them, but most breeders do not turn them during storing.

Incubators

Numerous commercial incubators are available in different brands, models, and prices. Independent of the chosen model, it is important that the incubator is stable, able to monitor the inner temperature, and able to maintain it. For this reason, it is better to keep the incubators in an air-conditioned room with constant temperature and humidity.

Most incubators have a circulating air system. This means that the air is forced by a fan to move all around the incubator. In this way the temperature is more uniform within the box. However, different ideas and concepts are constantly developing on AI, some of which seem very promising.

If we look at incubation under natural conditions, eggs are not incubated at a uniform temperature. Most hens develop what is normally referred to as the “brood patch” on the abdomen. When the hen is sitting on eggs, there is always a thermal gradient between the lower and upper parts of the egg under the hen. When the environmental temperature varies, the egg temperature will vary, too. This will cause a contraction and expansion of the egg content. These contractions and expansions should promote and improve breathing and metabolism of the egg and embryo.

One of the new concepts is to design incubators with a broad heater on the ceiling of the unit (imitating a brood patch); the eggs are kept on trays at the bottom of the box. In this way, the normal physiologic temperature fluctuations are respected, and the eggs should perform better. It is still too early to have a final opinion on the incubators built this way and on whether they perform better than “standard” incubators. However, the general feeling is that they do well. There are currently large professional breeding centers that are using such incubators.

Other parameters to evaluate when selecting an incubator are the following:

- Inner (usable) size
- Type of trays and their versatility
- Easiness for cleaning and maintenance
- Easiness for using
- Incubator mass (bigger the mass of the incubator, higher the stability of temperature and humidity)
- Availability of technical assistance.

There are several reasons to have more than one incubator on hand. Bird keepers incubating eggs from different species should have several devices. If this is not possible, the breeder should have at least two incubators, plus a good hatcher, for the following reasons:

- There will be eggs of different sizes
- Eggs with different numbers of pores per cm²
- Broken, altered, misshapen and repaired eggs (risky eggs)
- Eggs with different needs in term of relative humidity (RH)
- Different numbers of turnings per day (smaller eggs need a higher number of turnings per day)
- One of the incubators may break down.

Please note:

At least 1 month before the start of the breeding season, it is always advisable to run the machines to verify that everything is working properly.

At least 2 weeks before egg laying starts, incubators must be started (they need time to settle properly).

Those two easy steps will avoid bad surprises when one really needs the equipment to work.

Regular maintenance of incubators includes a complete check of the different pieces of the units, such as the wet bulb, thermometer, hygrometer, timers, and the turning mechanism that moves the trays. Furthermore, the incubators should be cleaned and disinfected using nontoxic bactericidal products approved for eggs and incubators. At the end of the season, all the incubators (and brooders) must be cleaned carefully and fogged with a suitable disinfectant.

Practical artificial incubation

AI can be extremely satisfying for the bird fancier, especially because he/she will have complete control of the eggs: hatching and development of the chicks will depend on his/her skill, precision, and perseverance. It must be remembered that different bird species lay eggs with different needs and

incubation periods. Amongst the more commonly kept species, this may range from 18 days in the budgerigar (*Melopsittacus undulatus*), to 30 to 31 days in the kea (*Nestor notabilis*), to 35 days in the gyrfalcon (*Falco rusticolus*), to 42 days in the ostrich (*Struthio camelus*).

Temperature

Heat is the major player for embryonic development. Recommended incubation temperatures for most avian species range between 36.9° and 37.5° C. The best result in parrots and some birds of prey is normally achieved with 37.2 ± 0.1° C. As a general rule, the higher the heat, the faster the development, and the lower the heat, the slower the development. Too high temperatures will shorten incubation time, resulting in an incomplete absorption of the yolk, on the other hand, temperatures that are too low, can eventually kill the embryo.

Humidity

Humidity regulates the transpiration of the egg and the embryo, and helps with hatching time.

As a general rule, humidity is monitored using the Relative Humidity (RH) and is expressed as a percentage. There are several ways to regulate RH and reach or maintain it. Among the most common, it is possible to:

- Introduce into the incubator a small container with distilled water (most incubators have a built-in container for the purpose).
- Reduce the airflow inside the incubator.
- Put chicken eggs in the incubator to maintain the appropriate level of humidity. This technique works well, and it is maybe more physiologic than adding water to the cup;

Whatever the method, a wrong (especially too high) RH is one of the most common causes of embryonic death. Most aviculturists recommend an RH below 40% in the first half of the incubation and below 45% during the second half.

In the end, it is not very important what RH is kept: the real goal is the highest possible percent of eggs hatching. In this perspective:

1. the right humidity is the one that works in a specific incubator and in a given place, and
 2. the easiest way to verify whether the RH is right or wrong is to monitor the weight loss of the eggs during incubation.
- *From the day an egg is laid until internal pipping, the egg should lose about 15% of its initial weight.*
 - *Monitoring the egg weight (and so the weight loss) during incubation is the easiest (almost the only) way to understand whether we are doing it right.*
 - *It is possible to divide the incubation time in thirds and weigh the eggs four times (day 0, 1st , 2nd, and 3rd period). Some eggs have to be weighed more often to ensure the desired weight loss.*

If weight loss differs too much from what is expected, RH should be adjusted, or the eggs moved to an incubator with a different RH.

Egg positioning and turning

Turning is another crucial factor for good embryonic development. If the eggs are turned less than they need, or they are not turned at all, the embryo can adhere to the eggshell from inside. In that case, we will observe a malpositioned chick.

There are basically two ways to turn the eggs:

- Rotation: rotating incubators have trays with rollers of different size and the eggs are positioned horizontally; when the rollers move, the eggs will turn over them. (typically used in parrot eggs)
- Tilting: eggs are positioned vertically with the air chamber up (typically used in galliform eggs). Tilting trays lean 45 degrees and have a 90 degree movement.

Candling

Candling is the only way to evaluate egg fertility and to estimate the age of the embryo.

Light candling, or "Classical candling". This is done in a darkroom. The egg is brought near a light source to transilluminate the shell. Some typical features of the egg will tell the experienced operator whether the egg is fertile and the approximate age of the embryo. Fertility can be assessed after 4 to 7 days of incubation, depending on the species, when ramified blood vessels are clearly visible.

Electronic Candling: in the past decade, digital or electronic monitors have been available on the market. Most of these devices work using infrared transmitters and sensors and doppler technology. They will amplify the cardiovascular signal of an embryo in the egg, allowing the breeder to detect the heartbeat of the embryo just a few days after incubation has started (e.g., Buddy, Avian Biotech International, Tallahassee, Fla., USA). Although these devices work well and are actually able to give good information, they should not be considered a total replacement for the standard, light candling, but instead be used as a device complementary to the classical technique.

Hatch and assisted hatch

The hatching process begins about 48 to 72 hours before the expected hatch day. This is the right moment to place the eggs in the hatcher and, much better, in a separate unit. Good record keeping and knowledge of incubation length will help to understand when the process really starts.

Most aviculturists recommend setting the hatcher at a temperature slightly lower than in the incubator (37.0° to 37.1° C) and at a humidity slightly higher (more than 50% relative). Whatever the settings in the hatcher, the eggs will be placed horizontally and will not be turned. It is highly recommended to place each egg in a single container (a plastic cup or similar).

The chick should start breaking the eggshell 24 to 36 hours after breaking the air chamber membranes or internal pipping. From that time on, the chick will breathe fresh air from outside the egg and will start vocalizing. If 36 hours after the internal pipping there are no cracks in the eggshell, then a hole will be made in the shell, over the air chamber, so that the chick can breathe. In the majority of cases, this is enough for a successful, natural hatch.

The time interval from pipping to hatching varies between species. On average, an interval of 24 to 72 hours is considered normal. During this time, the chick turns to break the shell and come out of the egg. If after this time there is no sign of movement, or if the chick is calling loudly (or worse is not calling at all), it is better to act (assisted hatch). Use the electronic heart monitor Buddy to assess cardiac function.

How to Proceed for Assisted Hatch

1. Maintain the egg in the same position of incubation.
2. Using a sterilized needle or fine drill bit, drill a hole over the air chamber (blunt egg point).
3. Using small tweezers, take away small parts of eggshell until the hole is big enough to allow for good visualization of the chick (likely wrapped in the membranes).
4. Use a cotton applicator, or a small paintbrush soaked with distilled water or sterile saline solution, to moisten the membranes.
5. If there is a net of small blood vessels, the chick is not ready to hatch. In this case, place it back in the hatcher and WAIT!
6. If blood vessels are not visible, it is better to break the membranes using a soaked applicator and to try to locate the bill of the chick.
7. Repeat the same action after 1 hour, widening the hole on the membranes and maintaining them moisturized.
8. Maintain the chick moist, especially around the bill.
9. Stimulate the chick to move and to free itself from the membranes with a gentle pressure of the applicator. At this point, vocalization is a good sign!
10. Always check the area around the umbilicus before freeing the chick completely.
11. When the chick is out of the eggshell, it is necessary to ligate and cut the umbilical cord.
12. Disinfect the navel and leave the chick in the hatcher for few hours to dry.

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FLAMINGO CRISIS- MEDICAL CHALLENGES IN RAISING LESSER FLAMINGO CHICKS

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Lesser flamingos (*Phoenicopterus minor*) are near threatened and only four active breeding sites are currently identified around the world. Etosha pan in Namibia, Sua pan in Botswana, Lake Natron in Tanzania and Kamfers dam in South Africa. Due to high environmental temperature and broken pipelines the Kamfers dam dried up in mid February 2019 in the middle of the flamingo breeding season, leaving thousands of day old chicks in a fight for survival. A huge rescue operation was initiated and flamingo chicks were moved first to the SPCA and translocated to zoo, rehabilitation centers and veterinary hospitals in South Africa. With the help of local and international volunteers and experts, the flamingos have been hand reared in an effort to re-release back into the wild. Correct initial stabilisation, treatment and husbandry at the site of collection prior to transport improves survival of the chicks. Metabolic bone disease, crop stasis, crop infection and pox lesions were the most common presentations seen in the chicks. Pox lesions were seen on the legs, eyes and beak of the chicks and vary from raised lesions with a diameter of several millimetres to invasive locally proliferate lesions with a diameter of several centimetres. Weight gain has averaged approximately 8 % in the first 3 weeks. Weight gain is an important indicator of the chick health status. The weight at release is a predictor of survival chances of the chick.

BLOOD COAGULATION ASSESSMENT OF CAPTIVE ASIAN ELEPHANTS (*ELEPHAS MAXIMUS*) USING VISCOELASTIC POINT-OF-CARE UNITS

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The leading cause of mortality among juvenile Asian elephants (*Elephas maximus*) under human care is elephant endotheliotropic herpesvirus (EEHV) (FUERY et al., 2018). As the disease progresses, widespread vascular damage, haemorrhage, and potentially death occurs (KOCHAGUL et al., 2018). The purpose of this study was to evaluate the effect of analytical variables (effect of time, activator, repeated pipetting, addition of sodium citrate, and 120 mile transport) when using three viscoelastic point-of-care units to assess Asian elephant blood coagulation. Blood from six healthy (EEHV PCR negative at the time of the study) adult (16-51y) Asian elephants was collected from the ear vein using vacutainers, preserved at room temperature, and evaluated at 0.5, 1, 2, 4, and 24 hr post collection using thromboelastography (TEG™), rotational thromboelastometry (ROTEM™), and dynamic viscoelastic coagulometry (Sonoclot™). All units allowed the assessment of coagulation. As time progressed citrated blood became more hypercoagulable, although, the use of new tubes for each time point led to less substantial changes up to 4 hours. Repeat pipetting led to significant hypercoagulability. Native fresh blood was hypocoagulable with weaker clot formation when compared to citrated blood at 30 min. Transported citrated blood sample at 24 hours had significant differences when compared with 30 min citrated blood sample. Kaolin and human tissue factor on Sonoclot™ produced a narrower range of clotting variables when compared to glass bead, suggesting that these activators might be more clinically useful. The information provided by this study will allow for methodology optimisation for future studies.

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RECOMBINANT HUMAN TISSUE FACTOR AND KAOLIN-ACTIVATED THROMBOELASTOGRAPHY (TEG) IN CLINICALLY HEALTHY ADULT ALPACAS (*VICUGNA PACOS*)

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Thromboelastography (TEG) is a rapid, reproducible test of haemostatic function in whole blood used to identify and quantify alterations of both hyper- and hypocoagulative disease states. TEG may have diagnostic potential in New World Camelids with hemostatic disorders. The study objective was to establish clinically applicable normal values using both recombinant human tissue factor (rTF) and kaolin-activated TEG methodologies for reaction time (R), clotting time (K), angle (α), maximum amplitude (MA), and time to maximum amplitude (tMA) of TEG from healthy male and non-pregnant female Huacaya alpacas (*Vicugna pacos*). Ten millilitres citrated whole blood from 20 clinically healthy adult alpacas (2-10 years) was collected by direct venipuncture. TEG analysis (rTF and kaolin-activated) was performed in duplicate, 30 minutes after collection. Prothrombin time (PT) and partial thromboplastin time (PTT) were obtained concurrently. All data were compared using Wilcoxon or t-test analyses based on data normality, with $P < 0.05$ considered significant. Mean values of TEG parameters (\pm SD) for adult alpacas using rTF and kaolin-activated methodologies were respectively: R= 6.99min (2.15), 7.53min (1.44); K= 3.43min (1.13), 3.82min (1.15); α = 48.51° (9.17), 45.06° (9.24); MA= 52.05mm (8.12), 53.15mm (8.08); tMA= 28.1min (3.8) and 32.1min (3.0). Mean PT and PTT were 8.6s (0.6) and 16.2s (2.3). Aside from tMA, in which rTF was faster ($P=0.001$), no significant differences in TEG parameters were observed between methodologies. Compared to reported rTF-activated TEG results in healthy dromedary camels, alpacas show a relatively smaller R, angle and MA. Species-specific normal values are, therefore, necessary for the interpretation of thromboelastography.

FAECAL INFLAMMATORY BIOMARKERS IN CAPTIVE TIGERS (*PANTHERA TIGRIS*)

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Non-invasive gastrointestinal health indicators are particularly interesting for zoological collections. Two faecal inflammatory biomarkers, N-methylhistamine (NMH) and S100A12, were measured for the first time in seven zoo-housed tigers (*Panthera tigris*) undergoing a randomised cross-over feeding trial. Baseline diet (BD) contained 8% boneless chicken, 3% degutted whole rabbit (DWR), 7% horse shanks, 82% supplemented horse-meat (HM). Experimental Diet (ED) contained 80% HM, 20% DWR. Control Diet (CD) comprised 100% HM. Both ED and BD were fed for 8 weeks without washout period. Faecal samples were collected over three consecutive days during weeks 0, 8 and 16. Faecal biomarker concentrations were measured using gas chromatography for NMH and in-house EIA for S100A12. Data were analysed with WRS2 package in R version 3.3.0. A repeated measures ANOVA showed a trend towards significance for faecal concentrations of NMH across dietary treatments ($F_t(1.25, 4.98) = 4.53, p = 0.084$). However, pairwise comparisons with adjusted p -values between diets failed to reach statistical significance. Concentrations were not significantly different for S100A12, ($F_t(1.11, 4.44) = 1.37, p = 0.306$). The results observed for NMH concentrations (BD > ED > CD) are at odds with significantly increased stool firmness ($p = 0.031$) in tigers fed ED. Increased concentrations of both markers have been associated with chronic enteropathies in domestic dogs. However, validation of both assays is still required for tigers, while existing for domestic dogs and cheetahs. Our preliminary findings indicate further investigation is warranted to determine their efficacy as biomarkers of gastrointestinal inflammation in tigers.

ADVANCED DIAGNOSTIC OPTIONS FOR RENAL DYSFUNCTION IN ASIAN ELEPHANTS (*ELEPHAS MAXIMUS*)

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Renal function is difficult to assess in elephants since blood kidney parameters may remain within reference ranges despite ongoing disease. We report 7 cases of renal disorders in Asian elephants, 4 of which were terminal.

Common presenting complaints included weight loss, intermittent oedema (abdominal, maxillary) and muscle fasciculations/convulsions. At later stages, pale mucous membranes, generalized weakness and polydipsia developed. In four terminal cases, histopathology confirmed chronic interstitial nephritis accompanied by lesions such as tubulo- or glomerulonephritis and mineralisation; cystic degeneration; or pyelitis.

In early stages, haematology and biochemistry alone did not qualify as sensitive diagnostics in cases confirmed histopathologically. However, hypercalcemia, dysproteinemia and anemia consisted common findings. BUN was the first of the renal enzymes to rise during course of disease, while serum Creatinine (SCrea) invariably remained unchanged until end-stage disease. In two elephants, after initial rise, BUN and SCrea even dropped dramatically prior to euthanasia.

Lowered urine creatinine (UCrea) consistently was the most reliable clinicopathologic indicator of underlying renal disease. UCrea remained significantly lower (mean \pm SD: 0.09 \pm 0.04 mg/ml; $n=7$) compared to unremarkable controls from the same facilities (0.26 \pm 0.08 mg/ml; $n=5$).

All affected elephants displayed ultrasonographically visible kidney lesions. Transrectal ultrasonography revealed abnormal kidney outlines and size ($n=3$), renal cysts ($n=5$), prominent renal vasculature ($n=5$) and/or hyperechogenicity of the parenchyma with distal acoustic shadowing ($n=5$).

We indicate that an approach not limited to blood analysis but including measurements of UCrea and ultrasonography is beneficial to assessing kidney function and identifying chronically ill Asian elephants.

URINE AS A PREDICTOR OF CARNIVORE HEALTH

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In many zoological institutions, anaesthesia is needed to obtain blood samples from carnivore felids, canids, and ursids. Anaesthesia can be a risk for these groups of animals and training for blood draws may not always be an option. Urine is easier to collect and can give a lot of valuable information. Our hypothesis was that urine chemistry values could help determine the health status of organ systems and predict blood chemistry values. Urine was collected from 30 zoo carnivores and placed in a no additive tube. Collection was performed via cystocentesis while the animal was anaesthetized, or from a sterile bowl or crate the day before or after anaesthesia, when cystocentesis was unsuccessful. Blood samples were obtained under anaesthesia and placed in lithium heparin. The urine and blood were both then refrigerated until processed, which occurred on the same day as collection, on Abaxis Vetscan® VS 2 (Abaxis, California, USA) using the mammal comprehensive diagnostic profile and large animal rotor. The urine was also submitted for a urinalysis, microalbuminuria, and urine protein creatinine ratio. The blood was submitted for complete blood count, serum chemistry and symmetric dimethylarginine test. Comparing the blood values to the urine values the urine was able to predict values for glucose, sodium, aspartate aminotransferase, alanine aminotransferase, amylase, and albumin. It is recommended to obtain the cleanest urine sample possible from carnivores at the same time a blood sample is collected to use as a reference for future urine samples to be used during clinical situations.

ACUTE PHASE REACTANTS IN HEALTHY, SICK AND DEVIL FACIAL TUMOUR DISEASE-AFFECTED TASMANIAN DEVILS (*SARCOPHILUS HARRISII*)

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The Tasmanian devil (*Sarcophilus harrisii*), is endangered in the wild due to the spread of Devil Facial Tumour Disease (DFTD). These tumours are infectious via allograft transplantation resulting in near 100% mortality. Tumour proliferation is uncontrolled due to evasion of the devil immune response. Reports of incubation periods of up to 15 months have resulted in prolonged quarantine of captive animals (HOGG and BELOV, 2019).

Changes in serum concentration of acute phase reactants (APR) can serve as sensitive, but non-specific, indicators of systemic inflammation, and assays have been widely applied in veterinary medicine. Baseline concentrations and inflammatory responses vary across species and therefore require investigation (BERTELSEN et al., 2009). The aim of this study was to conduct preliminary investigations into selected APR in devils, using commercially available domestic animal and human assays. Sera from 50 apparently healthy individuals were used to calculate reference intervals, and as a baseline to test the hypothesis that devils with DFTD, or other inflammatory conditions, would have decreased albumin and iron, and elevated serum amyloid A, C-reactive protein and haptoglobin concentrations.

Albumin concentration decreased in both non-DFTD and DFTD diseased devils, while haptoglobin increased in DFTD devils. Interestingly, while little or no inflammation is seen on a microscopic level in DFTD tumours, this study indicates that a systemic inflammatory response is produced. Longitudinal sampling of healthy and DFTD devils is required to investigate the onset of the systemic inflammatory response, and the association with other biomarkers and disease severity. Measurement of APR may also be useful in assessing future novel treatment protocols.

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REPTILE INFECTIOUS DISEASES WITH A FOCUS ON EUROPE

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Introduction

Infectious diseases play an important role in reptile medicine and an increasing number of potential pathogens has been described in recent years. The relationship between these infectious agents and disease is often complicated and not well understood and can depend on multiple factors. Pathogens with apparently increasing prevalence in wild and captive reptiles in different parts of the world include fungi (e.g. *Ophidiomyces ophiodiicola*) (ALLENDER et al., 2015; LORCH et al., 2016) and viruses (e.g. viruses in the order *Nidovirales*) (O'DEA et al., 2016; STENGLEIN et al., 2014; ZHANG et al., 2018). While the international trade in pet reptiles has influenced the spread of infectious agents as well as of their hosts, there are still distinct differences in the types and prevalences of pathogens in Europe compared to other parts of the world. Types and availability of diagnostic testing and the focus of research projects also influence our understanding of infectious diseases in these animals. This presentation will provide an overview of some of the most important infectious agents affecting reptiles commonly found as pets or in zoos in Europe, as well as presenting some more recently discovered pathogens.

Select Pathogens

Viruses: Viruses commonly found in reptiles in Europe include herpesviruses and picornaviruses in chelonians, adenoviruses and reoviruses in squamate reptiles, nidoviruses in snakes, and reptarenaviruses in boas and pythons. All of these have predominantly been found in captive reptiles, and their prevalence among wild reptiles is unknown. Several ranaviruses have been found in both wild and captive reptiles in Europe, as well as in amphibians and fish. There are many other viruses that are also found regularly in reptiles in Europe, but with less frequency.

A wide variety of herpesviruses have been described from chelonian hosts. In Europe, they are most commonly found in tortoises, especially *Testudo* spp. (KOLESNIK et al., 2017). The most common strains are testudinid herpesvirus (TeHV) 1 and 3. Herpesviruses are also found regularly in aquatic turtle species. Picornaviruses in the genus *Torchivirus*, also known as virus "x", have been described in European tortoises since the 1990's (MARSCHANG and RUEMENAPF, 2002). They are most often found in spur-thighed tortoises (*Testudo graeca*), but can also infect a wide variety of other tortoises (FARKAS et al., 2015; MARSCHANG et al., 2016). They have been shown to cause nephropathy and osteodystrophy in juvenile tortoises (PARIES et al., 2019).

Adenoviruses of the genus *Atadenovirus* are among the viruses most frequently detected in lizards and snakes (MARSCHANG, 2019). They are particularly common in bearded dragons. They mostly affect the gastro-intestinal tract and the liver. Atadenoviruses are hypothesized to have co-evolved with squamate reptiles (HARRACH, 2000; PÉNZES et al., 2009). There are also a wide range of adenoviruses that have been found in chelonian hosts, although less frequently than in squamates.

Nidoviruses have been shown to be an important cause of respiratory disease in various python species (STENGLEIN et al., 2014; DERVAS et al., 2017; HOON-HANKS et al., 2018). They have also been detected in boas in rare cases (MARSCHANG and KOLESNIK, 2016) and related viruses have been

detected in shingleback lizards with respiratory disease in Australia (O'DEA et al., 2016) and in wild Bellinger turtles suffering a severe disease outbreak in Australia (ZHANG et al., 2018). Since their discovery, detection rates in captive snakes in Europe have remained high, and nidoviruses are among the most common pathogens detected in pythons.

Reptarenaviruses are the cause of inclusion body disease (IBD) in pythons and boas (STENGLEIN et al., 2017). They are very frequently detected in boas, but detection in pythons can be challenging, and the virus is often only found in the brain, making detection in live animals difficult to impossible. Connections between virus infection, development of inclusion bodies, and clinical disease are not well understood. The reptarenaviruses have been shown to be genetically highly variable, which can complicate virus detection, and this has been hypothesized to play a role in disease development (HEPOJOKI et al., 2015).

Bacteria: Reptiles can carry a wide variety of bacteria as part of their normal flora, many of which are potential pathogens. Among the most commonly discussed of these are *Salmonella*, due to their zoonotic potential. In chelonians, mycoplasma are an important cause of respiratory disease and are very common in some tortoise species. Especially high prevalences have been found in Russian tortoises (*Testudo horsfieldii*) (KOLESNIK et al., 2017). Other bacterial pathogens found in reptiles include *Devriesea agamarum*, which is associated with skin lesions mostly in spiny-tailed lizards (*Uromastix* spp.), chlamydia, and mycobacteria.

Fungi: A variety of fungi are known to cause disease in reptiles. These include fungi in the genera *Ophidiomyces* in snakes and *Nannizziopsis* and *Paranannizziopsis* in various lizards. Related fungi have also recently been described in chelonians, but not yet in Europe. These fungi are most often associated with skin lesions in affected animals. *Metarhizium* spp. originally described as *Chamaeleomyces* spp. have been associated with granulomatous lesions on the tongue, as well as disseminated disease in various reptiles, mostly chameleons (SCHMIDT, 2015; KEPLER et al., 2014). A variety of other fungi have been described in reptiles, and are generally only associated with disease in immune compromised animals.

Ophidiomyces ophidiicola is the cause of snake fungal disease (SFD) and has been described as an emerging pathogen (ALLENDER et al., 2015; LORCH et al., 2016). While multiple studies have dealt with the prevalence of this pathogen in wild snakes in north America, the prevalence and importance of this fungus in Europe is less well documented. Sporadic cases have been reported in captive snakes in several European countries (SIGLER et al., 2013; PIQUET et al., 2018), and a recent study of wild snakes in Great Britain and the Czech Republic documented the occurrence of the pathogen in both countries (FRANKLINOS et al., 2017). Additional work is necessary to better understand the implications of these findings for both wild and captive snakes in Europe.

Conclusions

In many cases, disease associated with each of these types of infectious agents will depend not only on the pathogen itself and the host species, but also on environmental temperature, hygiene, and general husbandry as well as, in many cases, the presence of other infectious agents. Some are also very slow to cause disease, and animals may remain clinically inapparent carriers over extended periods of time. It is important to understand not only what pathogens might infect a given species, but also how animals can become infected, how the pathogens are shed, how long they can persist in the environment, and what options are available for pathogen detection in order to develop and optimize programs for risk reduction.

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POST MORTEM FINDINGS IN EIGHT SPECIES OF CAPTIVE CAECILIAN (GYMNOPHIONA) OVER A TEN-YEAR PERIOD

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Between July 2007 and June 2017 there were 122 individuals of eight species of caecilian present at some point in the Zoological Society of London's (ZSL) London zoo's collection, of which 86 died or disappeared (assumed died and scavenged). The mortality rate (deaths per animal-year at risk) ranged from 0.03 in the Congo caecilian (*Herpele squalostoma*) to 0.85 in Kaup's caecilian (*Potomotyphlus kaupii*). Among the 73 individuals examined *post mortem*, no cause of death or primary diagnosis could be established in 35 cases, but of the others the commonest cause of death was dermatitis (22 cases) with three cases of chytridiomycosis also presenting with skin lesions. When all significant pathological findings were considered, skin lesions of varying types were again the commonest (56 cases), particularly among the aquatic species: *Typhlonectes compressicauda* (18 out of 21 cases), *T. natans* (8/10) and *P. kaupii* (12/14). Mixed bacteria were consistently cultured from affected skin, with a predominance of *Aeromonas hydrophila*, whilst some individuals had acid-fast bacilli, nematode infections, skin-dwelling mites or achlorophyllous algae; all of which could initiate skin damage and/or invade opportunistically. Other common findings were poor gut-fill (35 cases), kidney and gastrointestinal lesions (10 cases each), generalised congestion (eight), poor body condition (six) and oedema (ascites and anasarca; six cases). This study adds to the growing body of knowledge about these poorly known and understood vertebrates and we hope will encourage further research into causes of their diseases and improvements in their health and welfare in captivity.

COMPARISON OF THE EFFECT OF ALFAXALONE VS MEDETOMIDINE AND KETAMINE FOR INDUCTION OF ANAESTHESIA IN *TRACHEMYS SCRIPTA* SP. UNDERGOING STERILISATION

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This prospective randomized study was approved by the Animal Ethics Committee of VetAgro-Sup and is registered under n°1721. The objective of the study was to compare the effects of two intramuscular anaesthesia protocols in *Trachemys scripta* sp. undergoing sterilisation. Forty-three healthy female red-eared turtles were premedicated with 1.5 mg/kg morphine subcutaneously (S.C) two hours before anaesthesia induction. The turtles were randomly assigned to receive either medetomidine (Dormilan, Axience) 0.2 mg/kg and ketamine (Anaestamine, Osalia) 10 mg/kg (group MK n=23) or alfaxalone (Alfaxan, Dechra Veterinary Products) 20 mg/kg (group A n=20) intramuscularly (I.M) followed by 2 mg/kg bupivacaine S.C. in the prefemoral fossa along the incision site. Anaesthesia was maintained with the administration of isoflurane (3%, adjusted according to a neuro-depression score) delivered into oxygen (100%) via an endotracheal tube. A pain score was assessed. Heart rate, muscle relaxation time (T_{MR}), tracheal tube insertion time (T_{TTI}), surgical incision time (allowed by an adequate anaesthetic depth corresponding to a neuro-depression score lower than 3) (T_{INC}) and recovery time (T_{REC}) were also recorded. Results and p-values were obtained using a T-test after logarithmic transformation of times.

Muscle relaxation time, T_{TTI} , T_{INC} were respectively 4.5, 2.8, 1.7 smaller in A than in MD (respective p-values of 1.5×10^{-10} , 3.2×10^{-8} , 9.705×10^{-4}). It was not possible to highlight a significative effect of the protocols on the full recovery time because of the variability of the surgery length. Alfaxalone and medetomidine combined with ketamine administered intramuscularly both provided a safe and smooth anaesthesia but alfaxalone enable a faster induction and led to a more reliable anaesthesia.

EPIZOOTIC MYCOBACTERIOSIS IN COLORADO RIVER TOADS (*INCILIUS ALVARIUS*) – PATHOLOGY AND CLINICAL CHALLENGE

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Mycobacteriosis is an important source of morbidity and mortality in many amphibian species, and epizootics have been attributed to research facilities (MILLER and FOWLER, 2012). Here, we report an epizootic in Colorado river toads (*Incilius alvarius*) that occurred in Berne Animal Park. Ten toads were imported from the U.S.A. in 2001. The first documented, captive breeding occurred in 2005 (SCHILDGER et al., 2006), and was replicated in 2012. Due to facility reconstructions, the juveniles of the second spawn were temporary kept under inadequate conditions with poor water quality, which led to mass morbidity and mortality in 2014 within three months (n=45). Clinical symptoms included lethargy, lameness and neurological symptoms such as tremor and paresis. From 2012 to 2018, 65 toads were sent for pathological examination. Twenty-nine (44%) were affected by Mycobacteriosis. Granulomatous lesions were mainly found in spleen (79%) and liver (62%), but also spinal cord/brain (28%), vertebral column/skull (24%) and coelom (24%) were regularly affected. Culture and species identification using MALDI-TOF MS revealed *Mycobacterium marinum* as the causative agent. In 2018, the remaining clinically healthy toads were screened by means of x-ray, coelomic ultrasound and endoscopy. In 8/15 individuals, ultrasound findings in the liver (e.g. hypoechoic masses) and endoscopic alterations (e.g. diffuse discoloration, vascular injection, rounded margins, protruding masses) led to the decision of humane euthanasia due to suspected mycobacteriosis. Postmortem examination revealed granulomatous lesions in 6/8 cases, and acid-fast bacteria in 4/8 cases. This study highlights the importance of follow-up examinations and surveillance after a mycobacteriosis outbreak.

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SEASON AND SEX SPECIFIC THYROID HORMONE LEVELS IN DIFFERENT EUROPEAN TORTOISE SPECIES (*TESTUDO* SPP.)

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Tortoises in the genus *Testudo* are among the most commonly kept reptiles in Europe and populations in the wild are listed as near threatened in CITES. It is therefore important to improve medical diagnostic testing for these species. Thyroid hormone levels play a major role in the regulation of various metabolic functions and thyroid dysfunctions have been described in several species. The goal of this study was therefore to establish specific reference intervals for the different sexes and seasons for tT4, fT4, tT3 and fT3 in Hermann's (*T. hermanni*) (255 samples), spur-thighed (*T. graeca*) (89), marginated (*T. marginata*) (72), and Russian (*T. horsfieldii*) (30) tortoises in order to improve diagnostic testing for thyroid diseases in these species. Total T4, fT4, and fT3 were measured using routine automatic immunoassay systems. Total T3 was measured by ELISA. Total T4, fT4 and fT3 were highest in spring and decreased in males to fall and in females to summer, except in male Russian tortoises, which had the highest fT3 levels in summer. The tT3 levels developed inversely and were lowest in spring. The results show significant differences ($p < 0.05$) between the seasons, sexes and species in tT4 and fT4. Total T3 and fT3 showed significant (< 0.05) variations between the different seasons and for the combination of species and season. This shows that thyroid hormones in tortoises are affected by several different factors. It is important to consider this when establishing reference intervals and evaluating thyroid hormones in sick tortoises.

CORNEAL LIPIDOSIS IN TWO COLLECTIONS OF LEMUR LEAF FROGS (*AGALYCHNIS LEMUR*)

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Corneal lipidosis is a recognized pathology in captive amphibians, particularly in aged females. Aetiology is not fully understood but may be related to diet, husbandry, and vitellogenesis.

The lemur leaf frog (LLF) (*Agalychnis lemur*) is critically endangered with the population having declined over 80% due to habitat loss and chytridiomycosis. Corneal lipidosis has been seen sporadically in LLFs housed at Bristol Zoo Gardens (BZG) and on occasion has resulted in globe rupture. Sixty-six LLFs underwent specialist ophthalmology screening. Corneal lipidosis was found in six animals, presenting as fine superficial axial/lateral paraxial crystalline stippling (2/6), localised dense lipid deposits in the lateral/perilimbal cornea (2/6), and generalised lipid deposition (2/6). The age of affected frogs ranged from eighteen months to five years. More severe generalised lipidosis occurred in older frogs. Lipidosis was bilateral in 4/6 frogs. Corneal vascularization was not present. Females appeared over-represented (5/6). Repeat screening six months later revealed two new cases in male frogs. Husbandry evaluation and screening has been performed on forty-five LLFs at another collection for comparison, showing one male and five females affected.

Corneal lipidosis in LLFs at BZG is more common in females and develops at a younger age. Initial Zootrition dietary analysis showed low cholesterol levels (0.08% DM), suggesting this is unlikely the sole contributing factor. Subsequent alterations to husbandry have included: reducing body condition, providing conditions for females with follicles to spawn and reducing the number of females in breeding condition. Annual screening is being performed to monitor prevalence and progression of disease.

ETORPHINE-AZAPERONE IMMOBILISATION FOR TRANSLOCATION OF FREE-RANGING MASAI GIRAFFES (*GIRAFFA CAMELOPARDALIS TIPPELSKIRCHI*) IN KENYA

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Etorphine-azaperone immobilisation was evaluated for translocation of Masai giraffes. Early reversal with low naltrexone doses (BURROUGHS et al., 2014; CITINO and BUSH, 2014) was analysed to assess benefits on physiological stability and handling safety.

Nine giraffes were darted with 0.012 mg/kg etorphine (Captivon 9.8 mg/mL, Wildlife Pharmaceuticals, South Africa) and 0.07 mg/kg azaperone (100 mg/mL, Kyron Laboratories, South Africa). Once ataxic, giraffes were roped for recumbency and restrained manually; Naltrexone (50 mg/mL, Kyron Laboratories, South Africa) at 3 mg/mg etorphine was given intravenously to reverse etorphine-related side effects. Protocol evaluation included physiological monitoring, blood-gas analyses, anaesthetic times and *ad-hoc* quality score scale (1 = excellent; 4 = poor).

Sedation onset and recumbency were achieved in 2.6 ± 0.8 and 5.3 ± 1.5 minutes. Cardio-respiratory function (HR = 70 ± 16 ; RR = 32 ± 8 ; SAP = 154 ± 22 ; DAP = 122 ± 16 ; MAP = 132 ± 16) and temperature (37.8 ± 0.5) were stable. Arterial gas analysis showed mild hypoxaemia ($\text{PaO}_2 = 67 \pm 8$ mmHg) and slight metabolic acidosis ($\text{pH} = 7.23 \pm 0.05$, $\text{PaCO}_2 = 34 \pm 4$ mmHg, $\text{HCO}_3^- = 12.9 \pm 1.2$ mmol/l). After 19 ± 3.5 minutes of immobilisation, giraffes were allowed to stand and loaded on trailer. Immobilisation quality was good, scored 2 (1 – 3); inductions and recoveries smooth, scored 1 (1 – 2) without excitatory phenomena. Renarcotisation didn't occur in 14-days *boma* follow-up.

The investigated doses provided partially reversed immobilisation with adequate physiological function that allowed uneventful translocation in free-ranging giraffes.

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THE BENEFIT OF SAND SUBSTRATE AND EXERCISE ON FOOT HEALTH IN ASIAN ELEPHANTS (*ELEPHAS MAXIMUS*)

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Foot problems are one of the most common health problems seen in captive Asian elephants (*Elephas maximus*) and prevention of these problems is of major importance for maintaining elephant welfare. Previous footcare measures followed mostly a curative approach and involved regular cleaning, inspection and trimming.

The current study collated pictorial data of Asian elephant foot health from 2000 to the present day and analyzed the observed differences in foot health before and after changing from a standard mixed concrete and natural soil ground to a complete deep sand substrate in 2011. Even grained, washed sand of 0.2 to 1.00 mm was used and sand depth ranged from 50 to 100cm. Enclosure sand was managed by thorough daily spot-cleaning and by regular turning over and reshaping.

Previous foot problems in this elephant herd included regular nail cracks, fluid-filled pockets or sole and nail abscesses requiring intensive remedial foot care. Since the substrate has changed to an even grained washed sand paddock in both the indoor and outdoor enclosures and extra exercise stimulating programs have been implemented, the incidence of foot problems has reduced markedly. Similarly, less time is now required for performing preventative foot care and veterinary interventions.

The present management of an elephant herd in an enclosure with deep even grained sand substrate, and the implementation of an exercise enrichment program documents the positive effect on Asian elephant foot health.

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HEMIMANDIBULECTOMY IN PYGMY SLOW LORIS (*NYCTICEBUS PYGMAEUS*): A TREATMENT OPTION FOR ADVANCED DENTAL DISEASE

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Captive slow lorises (*Nycticebus* spp.) have a high incidence of dental disease. In zoo specimen this is often diet related. In the illegal wildlife trade it is often seen after teeth are clipped or removed; the resulting secondary infections are a major cause of death in these individuals.

Two cases of advanced dental disease in captive Pygmy slow lorises (PSL – *Nycticebus pygmaeus*) from two zoological collections underwent hemimandibulectomy. The first case was an 8.5-year-old male PSL with a burst dental abscess and degloving injury of the left mandible. In the first of several surgeries the degloving injury was reduced in an attempt to save the mandible. A persistent infection with multidrug resistant bacteria led to wound breakdown and advanced osteomyelitis and hemimandibulectomy of the affected side was performed in two stages. Four months after initial presentation, the animal had fully recovered.

The second case was a 12-year-old female PSL presenting with right-sided facial swelling and dental disease. Despite extractions and antibiotic treatment, the facial swelling reoccurred one month later. Following antibiotic treatment based on extended culture and sensitivity, the diseased mandibular bone had fractured and hemimandibulectomy was performed. No post-operation complications were reported and the animal made a full recovery.

To the authors' knowledge, these are the first reports of hemimandibulectomy in PSLs, illustrating this is a viable treatment option for advanced dental disease in *Nycticebus* spp. This could have an impact on conservation efforts for animals rescued from the illegal trade, as well as for individuals living in zoological collections.

DECOMPRESSIVE SURGERY IN A RED FOX (*VULPES VULPES*) – REHABILITATION OF A PARAPARETIC WILD MAMMAL

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A non-ambulatory paraparetic adult male red fox (*Vulpes vulpes*) was admitted to the Wildlife Rehabilitation Center of the University of Trás-os-Montes e Alto Douro. An abscess was found in the thoracolumbar area, at the dorsal portion of the 13th thoracic (Th) vertebra. Spinal imaging, including radiographs and computed tomography, revealed comminuted fractures of Th13. These involved the pedicle and dorsal laminae of the vertebral body of Th13, and the respective spinous process. Left sided severe compression of the thoracolumbar spinal cord segment was identified. A modified decompressive surgery, consisting in removal of the compressive bone fragments, was performed based on what was previously described for dogs and cats.

Physical therapy, initiated 1 week after surgery, consisted in exercises to keep the animal standing and performing passive range of motion of all joints of both hind limbs. 7-weeks later, the animal was moved into an enclosure where different types of environmental enrichment were implemented and filmed in order to assess its progress. Scattering food throughout the enclosure resulted in increased movement and locomotion.

A wooden platform with steps was introduced, to allow a better stress-free evaluation of the animal's gait. Finally, after careful evaluation of the videos, the animal was considered fit for release.

This case suggests that rehabilitation of wild mammals with spinal cord compression can be successful and that different approaches to physical rehabilitation and neurological exam can be used, subjecting the affected animals to less stress.

A RETROSPECTIVE RADIOLOGICAL STUDY OF DEGENERATIVE ARTHROPATHIES OF CAPTIVE LARGE CATS: PREVALENCE, SEVERITY AND DISTRIBUTION

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Large cats live an estimated 25% longer in captivity than the wild. With longevity comes an increased incidence of degenerative joint disease (DJD). DJD is a welfare concern, yet can be difficult to diagnose and manage. Imaging of large cats can be challenging, with the appearance and significance of imaging findings unknown. To address these knowledge gaps, a retrospective radiological study was undertaken to determine prevalence, severity and distribution of arthropathies of lions, tigers, and cheetah imaged at 13 institutions between 1990-2017. A modified scoring system, based on the detection of domestic cat DJD, was applied to 404 studies. Appendicular joints and axial segments were scored as negative or positive, and a severity grade (mild, moderate, severe) was applied to positive sites. Data was analysed using a generalized linear mixed model with an underlying binomial distribution ($P \leq 0.05$). The effect of age, species, and site were assessed. There was a significant effect of age on the prevalence of arthropathy for both complete, and axial skeleton. The highest prevalence occurred in the axial skeleton of geriatric lions (88.7%, $n = 16$) and tigers (84.8%, $n = 8$) compared with cheetah (37.7%, $n = 22$). There was no effect of species or age within the appendicular skeleton. The prevalence of arthropathies in the appendicular skeleton of geriatric cheetahs was low (0%, $n = 15$). Axial arthropathies of lions and tigers were most commonly classified as severe and multifocal, compared with those of cheetah (mild, unifocal). Based on these findings, DJD of the axial skeleton of geriatric lions and tigers warrants further investigation.

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HOW TO BUILD A CHEAP INFRARED ANIMAL BEHAVIOUR MONITORING AND RECORDING SYSTEM USING A RASPBERRY PI TM

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Technological development has revolutionized the methods available to study and monitor animal behaviour, in many cases at moderately low cost. However, there is a paucity of cheap, off-the shelf systems for recording animal behavior in low-light situations. We developed a system for remote recording and monitoring animal behavior in visible and near infrared light based on the open Raspberry Pi TM computing platform. Raspberry Pi TM ® 3B computers were fitted with infrared-sensitive cameras (Raspberry PiNoir TM) (<https://www.raspberrypi.org/>), configured using a GNU/Linux operating system, and powered by a standard 5600 mAh USB battery pack. Lighting was provided with a single 850 nm 3W infrared LED powered by four alkaline AA batteries. The system was encased within an IP65 electric junction box, with a custom opening for the camera lens and IR LED. Total cost of the system was < EUR 75.

The system was tested during an overnight translocation of 8 dorcas gazelles in Senegal. The camera cases were affixed to the top of the wooden transport boxes, providing a top view for the cameras. The camera systems recorded an excess of 16 hours of continued video stream, providing high quality imaging of the gazelles at all times. Recordings allowed evaluation of respiratory rate, body posture, movement, and other behavior of the gazelles.

We have found the open Raspberry PiTM computing platform to be highly customizable and inexpensive. It can remotely record and monitor animal behavior in many zoo settings, such as animal transport, behaviour studies or nest monitoring.

EFFECTS OF 3T MAGNETIC RESONANCE IMAGING EXPOSURE ON THE BEHAVIOUR AND ORIENTATION IN MAGNETIC SENSITIVE SPECIES: HOMING PIGEONS (*COLUMBA LIVIA*) AND LOGGERHEAD SEA TURTLES (*CARETTA CARETTA*)

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We evaluated the behavioural effects after exposure to magnetic resonance (MR) on two species sensitive to magnetic field: homing pigeons and sea turtles.

A group of young pigeons (*Columba livia domestica*) were exposed to either a constant (no sequence) or a varying (echo planar sequence) magnetic field in a 3T MR unit for fifteen minutes, while a control group was not exposed to the MR field but otherwise shared all aspects of the procedure. All animals were released the next day from the same site, unknown to them, 15 km from the dovecot. A second release took place three weeks after this time 30 km from the dovecot. A set of variables was measured after each release. Of these, the only significant difference between groups was an increase of the variability in the angle of departure in those animals exposed to the variable magnetic field the day before release, suggesting at least a temporary interference with orientation.

In the case of the marine turtles, the anesthetized animals under MR showed a clear response to varying magnetic field, as evidenced by increasing heart rate and movements. Additionally, exposed animals showed changes in their swimming patterns for some days while in rehabilitation. A preliminary study found that these changes could be registered and characterized using motion daily-diary tags (recording tri-axial acceleration, depth and relative body position in the water).

Our results on homing pigeons and sea turtles show that diagnostic MR imaging might have temporary effects on the navigation of species with magnetoreception capabilities.

TRANSTHORACIC ECHOCARDIOGRAPHY AND CARDIAC BIOMARKERS IN HEALTHY CAPTIVE MALE AND FEMALE SQUIRREL MONKEYS (*SAIMIRI* SPP.)

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The purpose of this cross-sectional study was to investigate the feasibility of transthoracic echocardiography in healthy squirrel monkeys as well as to provide species-specific normal values for standard echocardiographic measurements. A secondary aim was to determine blood levels of cardiac biomarkers, N-terminal pro-brain natriuretic peptide (NT-proBNP) and cardiac troponin T (cTnT). Furthermore, a non-invasive, smartphone-based ECG (AliveCor Vet TM®) monitoring device was used to evaluate the heart rate and to diagnose possible arrhythmias. Data used in the study were collected during the annual health examination of the squirrel monkey colony, which includes physical examination and blood sampling under general anaesthesia.

In this study, two-dimensional (2D-), time-motion (M-) and Doppler mode echocardiographic variables were obtained in 14 squirrel monkeys. Transthoracic echocardiography was performed in right and left lateral recumbency. Similar standard right parasternal and left apical images were obtained as in dogs and cats and normal values for routine 2D, M-mode and spectral Doppler measurements were generated. Thirteen animals were considered healthy and one squirrel monkey was identified with significant aortic regurgitation and dilation and consequently excluded from the study. NT-ProBNP and cTnT concentrations were available for 7/14 monkeys with NT-proBNP concentrations below detection limit in all animals and a mean cTnT concentration of 0.049ng/mL. Electrocardiography was performed in 13/14 squirrel monkeys. A significant arrhythmia was diagnosed in the individual monkey suffering from significant aortic dilation and regurgitation.

This study presents echocardiographic normal values and additional cardiovascular data in anaesthetised squirrel monkeys, of value both in zoo animal health care as well as laboratory medicine, where the squirrel monkey may be used as an animal model.

ENDOSCOPIC RESOLUTION OF EGG-YOLK COELOMITIS IN BLACKCHIN GUITARFISH (*GLAUCOSTEGUS CEMICULUS*)

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Guitarfish (Rhinobatidae) are common species held in public aquariums for display. Although its reproductive biology is well described (CAPAPÉ and ZAOUALI, 1994), literature about their diseases is scarce (TUXBURY et al., 2017). In March 2018 a moderate coelomic swelling was noticed in a female adult Blackchin guitarfish (*Glaucostegus cemiculus*). Despite no mating being observed, gestation was suspected as the main cause, but an ultrasound examination did not reveal any foetal structures. Six months later, the swelling had increased and another ultrasound examination was performed. Marked uterine distension with hypoechoic fluid was observed at this time as well as moderate amount of slightly dense free fluid within the coelomic cavity. Radiographs were taken to evaluate the presence of foetal structures but none were visible. Treatment was initiated with antibiotics and vitamin C as an immunostimulant. After a month of treatment, coelomic swelling had increased and on ultrasound the density of the fluid had augmented showing a heterogenic appearance. A reproductive endoscopy was performed a few days later to better evaluate the uterine content, where a collapsed egg shell was found inside the left uterus but the endometrium appeared normal. Cannulation of the abdominal pores revealed a large amount of turbid yellow dense fluid which had no cellularity, and neither bacterial nor fungal growth was obtained on culture in specific media. After a presumptive diagnosis of egg-yolk coelomitis, coelioscopy under anaesthesia, with intravenous propofol at 2 mg/kg, was performed and several litres of the same fluid were drained during the procedure. Antibiotics and corticosteroids were administered during surgery and antibiotic therapy was continued for several weeks after. Currently the animal condition is normal and no recurrence has occurred.

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MONITORING OVARIAN CYCLICITY IN A CAPTIVE KOMODODRAGON (*VARANUS KOMODOENSIS*) BY ULTRASOUND EXAMINATION

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The reproductive cycle of a 9 year old female Komodo dragon was monitored weekly by transcutaneous ultrasound without using any restraint for over 4 years.

Main reasons for monitoring the reproductive cycle: (1) To define the best time for breeding introduction to avoid aggressive behavior. (2) To discover pathological changes in the reproductive organs in an early stage. Follicular stasis is the number one cause of death in female Komodo dragons.

Follicle diameter varied from 5 mm in the early development stage to 40-52 mm in pre-ovulatory follicles. One or two follicular growth waves were observed at each cycle prior to-ovulation. During the study period five ovulations were observed with an ovulation interval of ~1y. At age 9 – 11 years oviposition occurred 14-24 days after ovulation. From the age of 12 years the ovulation-oviposition interval became longer. The pre-ovulatory follicles were smaller in size and ovulated when at just 35 mm. Oviposition resulted in abnormal, infertile eggs. In the 3 cases when 2 follicular growth waves were observed, all large follicles of the first wave went into regression.

The male was introduced when the follicles were 35-40mm in size. Aggression during introductions remained at acceptable levels. During the final 2 years male interest decreased and no matings occurred. No pathological changes were observed during the study period.

In conclusion ultrasound monitoring of ovarian activities in unrestrained Komodo dragons is a valuable tool to predict best timing for breeding introductions.

GOING TO THE DOGS – GLOBAL CONSERVATION IMPACTS ON WILDLIFE FROM DOMESTIC DOGS

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Dogs were domesticated by humans some 15,000 years ago. They have been selectively bred for a range of behaviours [e.g. hunting, protection]. Dogs are generally associated with humans and their habitations but also range widely beyond these. The estimated one billion dogs worldwide exist on a gradient from free-ranging to wholly dependent on humans. As human populations grow, so do the associated dog populations. Dogs predominantly impact wildlife through direct predation. However, harassment, disease transmission, resource competition and hybridisation constitute important additional impacts. Dogs have been incriminated in the extinction of at least 11 species, and jeopardize the future of some 190 threatened species. The Wildlife Conservation Society works across 60 countries, and dogs impact wildlife conservation efforts in practically every one. Predation has been widely documented, affecting, inter alia: saiga (*Saiga tatarica*), and argali (*Ovis ammon*) in Inner Asia, as well as pudu (*Pudu pudu*) and Darwin's fox (*Lycalopex fulvipes*) in Patagonia. In northern Mozambique dogs are implicated as rabies and canine distemper virus reservoirs impacting the African wild dog (*Lycaon pictus*). Generally, dogs rarely become truly independent [feral] from humans, relying, at least to some extent, on human-associated resources. Research on the basic ecology of dogs in the various landscapes is lacking and thus is urgently needed. Due to their close association with humans, managing the impact of dogs on wildlife inherently constitutes an ethical dilemma. The varied and complex management options must recognize contrasting moral imperatives (conservation vs. welfare) and thoughtfully address necessary changes in human behaviours.

WILDLIFE CONSERVATION MANAGEMENT AT THE NEXUS OF VETERINARY SCIENCE AND ECOLOGY – THE DEVELOPMENT OF A NOVEL DOUBLE TAGGING SYSTEM ENABLING TRACKING OF ENDANGERED BLACK COCKATOOS IN WESTERN AUSTRALIA

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A novel tracking methodology was developed to gather data and address key ecological knowledge gaps in relation to habitat use and threatening processes, considered critical for the conservation management of Western Australia's three threatened black cockatoo species, Carnaby's cockatoo *Calyptorhynchus latirostris*; Baudin's cockatoo *C. baudinii*; and forest red-tailed black cockatoo *C. banksii naso*.

The development of the double-tag mounting protocol utilising GPS and satellite tags, and the attachment of the tags to the birds, which underpinned the ecological research, has involved collaboration between wildlife veterinarians and ecologists. This inter-disciplinary approach applying the diverse expertise associated with these discipline fields, has been fundamental to the success of the project.

The double-tag mount was trialled on captive black cockatoos and subsequently has been successfully used to track all three species of black cockatoos in the wild. Since 2015, our research team have successfully deployed 84 tagged birds, collecting over 140,000 location fixes, 33,000 km of track movement and over 2.8M accelerometer records. These data are being used to address key research objectives in species' recovery plans, and to inform conservation management of the threatened black cockatoo species in modified urban, industrial and agricultural landscapes.

SURPRISING BIODIVERSITY BETWEEN ZOO ENCLOSURES - A POTENTIAL INTERFACE TO VETERINARY CARE AND RESEARCH

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Zoo enclosures and areas in between harbour diverse communities of free-ranging animals and plants. In a 3-year survey (2005-2007) to assess free-living organisms (plants, fungi, animals) in the Zoo Basel, a team of 46 zoologists and botanists documented 3'110 species. Due to the lack of experts, not all taxonomical groups could be considered and therefore, the species diversity is estimated to may exceed 5'500. Thus, the number of free-ranging species is approximately eight to ten times higher than the actual zoo animal population of the Zoo Basel (11.6 ha city zoo with 646 species and 1 Mio visitors/y). Notably, the survey also detected a significant proportion of threatened species (18%) including 113 species listed on the Red Lists of Switzerland (Federal Office for the Environment). As an example, Zoo Basel serves as breeding site for the successful reintroduction of the white stork (*Ciconia ciconia*) in the Northwest of Switzerland. Approximately 25 breeding pairs are free ranging on zoo grounds and produce around 50 chicks per year. Zoo Basel's veterinarians perform medical interventions and research.

Veterinary activities between zoo enclosures have been traditionally focused on pest control. However, the findings of the survey and the example of the white stork show that biodiversity on zoo grounds offers an enormous potential for the collection veterinarian to become involved in wildlife conservation.

MYCOBACTERIUM TUBERCULOSIS COMPLEX DIAGNOSTICS IN ASIATIC LION (*PANTHERA LEO SSP. PERSICA*)

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Two Asiatic lions (*Panthera leo ssp. persica*) were exported from the Conservation Breeding Centre at Sakkarbaug Zoo, India, to ZSL Whipsnade Zoo, UK. This transfer was part of a collaboration between ZSL, Wildlife Institute of India (WII) and Gujarat Forest Department (GFD), of which one objective was improving the genetic viability of the European Endangered species Programme (EEP).

Pre-export disease screening was comprehensive but testing for infection with bacteria from the *Mycobacterium tuberculosis* complex (MTC) was not available in India.

After import the lions were housed in strict isolation. A single intradermal comparative tuberculin test showed a positive response in both lions. Sequential interferon-gamma tests on heparinised serum showed an increasing response to the PPDB and ESAT6 and CFP10 peptides. PCR on broncho-alveolar lavage and faeces was negative, and no lesions were observed on abdominal ultrasonography or thoracic radiography. An experimental test for the detection of MTC bacteria in blood using the phage-RPA technique (SWIFT et al., 2016) was positive. Three female lions at ZSL London Zoo, assessed as MTC-free, were treated as controls, all testing modalities yielding negative results.

The risk of assimilating these two newly imported lions into the EEP was deemed too high and euthanasia was performed. Sperm and oocytes were harvested prior to euthanasia for conservation purposes. No lesions were found at examination *post mortem*, and no bacteria from the MTC were cultured.

This case highlights the challenges interpreting ante-mortem MTC screening, especially where no validated tests exist. Using individuals assessed as disease-free can facilitate decision making and communication to stakeholders.

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TICK-BORNE DISEASE IN A CHANGING CLIMATE: INVESTIGATING THE EMERGENCE OF *BABESIA ODOCOILEI* IN CANADIAN CERVIDS

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Blacklegged ticks (*Ixodes scapularis*) are vectors for several important human and veterinary pathogens in North America, and the northern range of this tick is expanding due to anthropogenic influences on the environment and climate warming. The role for migratory birds in the epidemiology of non-zoonotic tick-borne animal diseases has received little research attention. *Babesia odocoilei*, a tick-borne haemoparasite endemic to the southeastern US, was first documented in Canada in 2012 as the cause of acute haemolytic anaemia in cervids. Clinical signs in reindeer (*Rangifer tarandus*) and wapiti (*Cervus canadensis*) include lethargy, haemoglobinuria, and sudden death. Parasitaemia is usually subclinical in white-tailed deer (*Odocoileus virginianus*). Following a disease outbreak in cervids at the Toronto Zoo (Ontario, Canada), this study was designed to investigate the prevalence of *B. odocoilei* in cervids and bird-borne ticks in Ontario, using PCR and DNA sequencing. Ticks were collected from northward-migrating birds ($n=1,102$) captured at a migration monitoring (bird banding) research station during 2016-2017. Prevalence of *I. scapularis* infestation was 3.2% in 2016 and 6.7% in 2017. Across both years, 0.2% of birds carried *B. odocoilei*-positive ticks, suggesting that migratory birds could potentially contribute to tick and pathogen range expansion. A prospective post-mortem survey investigated the prevalence of *B. odocoilei* infection in wild/farmed/zoo cervids ($n=270$) in 2016-2018. *Babesia odocoilei* was detected in clinically normal cervids: 1% (2/142) of farmed red deer (*Cervus elaphus*), 4% (3/68) of wild white-tailed deer, and 3% (1/29) of captive wapiti, indicating that these species may be reservoirs for *B. odocoilei* in Ontario.

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TRENDS AND INCIDENCE OF WEST NILE VIRUS INFECTION IN GOSHAWKS (*ACCIPITER GENTILIS*) IN A HUNGARIAN WILDLIFE RESCUE CENTER BETWEEN 2008 AND 2018

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The goshawk (*Accipiter gentilis*) is a rare visitor at the Wildlife Rescue Center of the Budapest Zoo composing only a few percent of the raptors admitted annually, though it is a protected species of the Hungarian fauna. Through our experience over the past 10 years, goshawks are not rewarding patients. Of the 29 individuals attended to over the past 10 years, only 2 (6.9%) were rehabilitated and released, 18 (62.1%) died at our facility, 3 (10.3%) were humanely euthanized and 6 (20.7%) recovered from disease or injury but were maintained in a captive setting. Of the 29 patients attended to over the past decade, 31% (n=9) were confirmed to have succumbed to lineage 2 West Nile virus (WNV) through immunohistochemistry, RT-PCR (LINKE et al., 2007) and genetic sequencing. Our cases comprise 30% of the cases reported in Hungary in this timeframe. Despite rigorous treatment through marbofloxacin, meloxicam and continuous i.v. supportive therapy, mortality was 100% in suspected individuals with an alarming progression of disease ranging from minor apathy upon admittance, to severe CNS signs and death in 1-3 days. Of these 9 cases, 4 (44.4%) presented in the past 3 years whilst in the other cases were distributed over the course of the remainder of the study period suggesting an increased incidence of the disease in the past years. Furthermore, this increase in the number of cases of WNV in goshawks follows a similar trend to the frequency of this ailment in both equine and human patients across the country.

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RESPIRATORY DISEASE IN CAPTIVE BONOBO (*PAN PANISCUS*)

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A group of 14 bonobos (born June 1978 – August 2017) suffered from respiratory disease in six outbreaks (February 2017 – January 2019) at Zoo Planckendael. A multiplex PCR for adenovirus (AV), coronavirus (CV), human metapneumovirus (hMPV), rhinovirus/enterovirus (REV), influenza A/B, parainfluenza (PI), respiratory syncytial virus (RSV), *Bordetella pertussis*, *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* was performed on 24 nostril swabs of 9 individuals. Twenty-three other nostril swabs (same 9 individuals) were used for bacterial cultures. PCR was positive for RSV in February 2017, REV in August 2017, AV and REV in December 2017, PI in July 2018, REV in December 2018 and CV and hMPV in January 2019. Nine bacterial cultures were positive for *Streptococcus pneumoniae*. The animals were treated with oral fluids and mucolytics, and oral or parenteral antibiotics in all 6 outbreaks. Only RSV and hMPV necessitated anaesthesia for the treatment of some animals. One 2-week-old baby with RSV was treated and hand-reared for 10 days in an incubator successfully. One 1.5-year-old infant died suddenly overnight due to pneumonia; lung PCR was positive for AV and hMPV; *S. pneumoniae* was cultured; the lung showed large congested areas and histopathology showed infiltration of macrophages, neutrophils and bacterial aggregates. Three animals aged 2, 15 and 24 years, needed anaesthesia for treatment with fluids, antibiotics, NSAIDs and corticoids. One 6.5-year-old was anaesthetised twice for the same treatment. These 4 animals recovered completely. RSV, hMPV and *S. pneumoniae* appear to be more pathogenic than the other detected infectious agents in bonobos, especially young animals.

COMPARISON OF THREE DIFFERENT IMMOBILIZATION PROTOCOLS IN SEMI-FREE RANGING KHULANS (*EQUUS HEMIONUS KHULAN*)

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Ultra-potent opioids are widely used for anaesthesia of non-domestic equids. Due to limitations regarding their availability and severe side effects, this study was designed to assess alternative protocols.

Due to management reasons, 43 anaesthesias were performed on 28 semi-free ranging khulans. Vital-parameters were assessed every 5 minutes.

The following dosages created reliable anaesthesias, without supplementation. EADB (n=6): etorphine^a (0.02 ±0.01mg/kg), acepromazine^a (0.05 ±0.02mg/kg), detomidine^b (0.06 ±0.01mg/kg), butorphanol^c (0.06 ±0.01mg/kg); KMMB (n=27): ketamine^d (3.16 ±0.53mg/kg), medetomidine^e (0.09 ±0.01mg/kg), midazolam^f (0.28 ±0.05mg/kg), butorphanol (0.18 ±0.04mg/kg); TZMB (n=7): tiletamine^g (1.36 ±0.22mg/kg), zolazepam^g (1.36 ±0.22mg/kg), medetomidine (0.09 ±0.01mg/kg), butorphanol (0.2 ±0.03mg/kg). Anaesthesia was reversed with atipamezole^h IM (5mg/1mg medetomidine or detomidine), naltrexoneⁱ IV (2mg/1mg butorphanol or 50mg/1mg etorphine), and flumazenil^j IV (0.3mg/animal), 60 minutes after induction. KMMB and TZMB provided significantly better muscle relaxation, while the EADB protocol showed significant faster recoveries. PaO₂ and SaO₂ were significantly higher for KMMB and TZMB with also a higher mean respiration rate, while the PaCO₂ was lower.

The opiate-based protocol is recommended for short-time procedures, whereas KMMB and TZMB provided reliable anaesthesias for prolonged procedures.

^aLarge Animal Immobilon, Novartis, Camberley, UK

^bEquidor®, Richter Pharma AG, Wels, Austria

^cAlvegesic®, Alvetra u. Werfft Animal Nutrition GmbH, Vienna, Austria

^dKetamidol®, Richter Pharma AG, Wels, Austria

^eMedetomidine 20mg/mL, magistral formulation, Vienna, Austria

^fMidazolam 50mg/mL, magistral formulation, Vienna, Austria

^gZoletil®100, Virbac, Glattbrugg, Switzerland

^hNarcoStop, Richter Pharma, Austria;

ⁱNaltrexon, Tsavran Pharmaceuticals, Cyprus, Greek

^jAnexate, Roche Pharma, Grenzach, Germany

EFFECTS OF THE PERIPHERAL α 2-ADRENOCEPTOR ANTAGONIST VATINOXAN ON MEDETOMIDINE-INDUCED BRADYCARDIA AND HYPERTENSION IN RED DEER (*CERVUS ELAPHUS*)

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Alpha-2-adrenoceptor agonists are commonly used for immobilisation of non-domestic mammals. However, cardiovascular side effects, such as bradycardia and hypertension, remain a major concern. We investigated the effects of an alpha-2-adrenoceptor antagonist, vatinoxan, on cardiovascular and sedative properties of medetomidine in red deer (*Cervus elaphus*).

Ten semi-freeranging animals (69.6 ± 10.3 kg; range: 53-89 kg) were anaesthetised twice in a randomised, crossover study with medetomidine^a (100 μ g/kg) and tiletamine/zolazepam^b (2.5 mg/kg) administered IM, followed by vatinoxan^c (100 μ g/kg) treatment or an equivalent volume of saline^d IV 35 minutes later. Baseline values, including heart rate (HR) and mean arterial blood pressure (MAP), were assessed 5 minutes prior to vatinoxan/saline treatment. The assessment was continued in 10 second-intervals for three minutes, 60 second-intervals for following 10 minutes and 5 minute-intervals for remaining 15 minutes after treatment. Anaesthesia depth was assessed before and after treatment.

HR increased significantly ($p < 0.001$, linear mixed-effects model) 10 seconds after vatinoxan injection by an average of 102 % with mean maximum HRs of 85 ± 10 beats minute^{-1} (range: 73-107) and remained significantly above baseline for 15 minutes. MAP significantly decreased ($p < 0.001$) by a mean decline of 34 % from baseline at vatinoxan injection with mean minimum values of 80 ± 7 mmHg (range: 68-89 mmHg) and remained below baseline until end of anaesthesia. MAP and HR remained constant after saline injection. Duration and intensity of anaesthesia were not influenced by vatinoxan. Vatinoxan reversed hypertension and bradycardia induced by medetomidine without causing hypotension or affecting quality of anaesthesia in red deer.

^aMedetomidine 20mg/ml, magistral formulation, Vienna, Austria

^bZoletil®100, Virbac, Glattbrugg, Switzerland

^cVatinoxan, Vetcare Finland Oy, Helsinki, Finland

^dIsotonic sodium chloride solution 0.9 % ad us. vet., B. Braun GmbH, Maria Enzersdorf, Austria

Acknowledgements

The authors would like to thank Vetcare for providing the experimental substance vatinoxan.

Ethical statement

All procedures and experiments were approved by the institutional ethics and animal welfare committee in accordance with the guidelines for good scientific practice, guidelines and regulations for animal care and for good scientific practice at the University of Veterinary Medicine, Vienna and national legislation. All experiments were conform to the Austrian Animal Experimentation Act (Tierversuchsgesetz 2012) and were approved from the animal experimental committee of the Federal Ministry of Science, Research and Economy (GZ: BMWFW-68-205/0191-WF/V/3b/2017).

COMPARISON OF ARTERIAL BLOOD GASES AND OTHER PHYSIOLOGICAL PARAMETERS BETWEEN TWO ANAESTHETIC PROTOCOLS IN SEMI-FREE RANGING SANCTUARY CHIMPANZEES

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Information is limited regarding differences in arterial blood gases (ABG), other physiological parameters, and anaesthetic quality between anaesthetic protocols in chimpanzees. Twenty-three healthy sanctuary chimpanzees were randomly allocated to two anaesthetic protocols during routine health checks: tiletamine/zolazepam (Zoletil® Virbac, France) 6 mg/kg (TZ; n=12, 9-25 years, 38.6-56.4 kg) or tiletamine/zolazepam 2mg/kg plus medetomidine (Medetomidine 10mg/ml, Kyron Laboratories, South Africa) 0.02mg/kg (TZM; n=11, 8-24 years, 26.3-63.9 kg). Supplemental ketamine (Ketamine 1g, Kyron Laboratories; 2 mg/kg) was required in 10 chimpanzees on TZ protocol. Physiological parameters were monitored every 3 to 5 minutes. ABG were measured at the beginning (T1=TZ 17'±4'; TZM 13'±2.5') and end of the procedure (T2=TZ 34'±4.1'; TZM 32'±2.8') using the iSTAT® analyser (Abbot Point of Care Inc, USA). Statistical comparison was made using unpaired t-test (P<0.05). TZM induction time was shorter (4±1 vs 7±3 minutes; p=0.003). TZ was associated with higher heart rate (HR; average 80±11 vs 67±12; p=0.016), lower blood pressure (BP; average mean arterial pressure 91±10 vs 99±20) and poor muscle relaxation. In both protocols T1 BP, HR and lactate values were higher than T2; The ABG trend was similar: initial hypoxaemia (partial pressure of oxygen and oxygen saturation=60±16mmHg, 88±8% for TZ; 54±5 mmHg, 87±11 % for TZM) resolved with oxygen supplementation. TZM recovery was shorter (55% < 1hr) than TZ (8%<1hr; p=0.024). Overall quality of anaesthesia was higher for TZM with no ketamine needed (p<0.001). Both protocols were adequate for immobilisation, however, TZM resulted in faster induction, better muscle relaxation, more stable anaesthesia and shorter recoveries.

THE IMPACT OF VATINOXAN ON A MEDETOMIDINE-BASED ANAESTHESIA IN PATAGONIAN MARAS (*DOLICHOTIS PATAGONUM*)

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α 2-adrenergic agonists are commonly used in veterinary medicine as sedative and analgesic drugs. In addition to their desired sedative effects in the central nervous system, the drugs lead to cardiovascular depression due to peripheral vasoconstriction. Vatinoxan, a peripherally acting α 2-adrenergic antagonist, reduces cardiovascular depression of α 2-adrenergic agonists in several species (HONKAVAARA et al., 2017; RESTITUTTI et al., 2018), without effacing the sedative effect. In a randomised, crossover study*, four male and five female captive Patagonian maras (*Dolichotis patagonum*) were anaesthetised with a combination of ketamine (5 mg/kg), medetomidine (0.1 mg/kg) and midazolam (0.1 mg/kg) with and without vatinoxan (0.8 mg/kg) injected intramuscularly. Oxygen was administered by face mask during the entire procedure. Vatinoxan significantly reduced arterial blood pressures measured from 20 to 40 minutes ($p=0.0007$). For example, the average mean arterial pressure (MAP) at 20 minutes was 81 mm Hg and 112 mmHg with and without vatinoxan, respectively ($p=0.0001$). Heart rate, respiration rate, end-tidal CO₂, arterial oxygen saturation measured by pulse oximetry, tissue oxygen consumption (arterio-venous oxygen content difference), and tissue (muscle) oxygenation as measured by near-infrared spectroscopy did not differ between treatments. Without vatinoxan, four animals were hypertensive (MAP>120 mmHg) while with vatinoxan four animals were hypotensive (MAP<60 mmHg). The latter probably could have been prevented through a reduction of vatinoxan dosage. The study confirmed the hypothesis that the dose-dependent increase in blood pressure induced by medetomidine can be reduced with vatinoxan. Interestingly, it failed to show if the reduction in blood pressure is physiologically beneficial through better perfusion.

*The study was conducted under a permit from the Danish Experimental Animal Inspectorate (permit #2017-05-0201-01219).

Acknowledgements

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CHALLENGES IN WILD BOAR (*SUS SCROFA*) ANAESTHESIA. COMPARISON OF TWO ANAESTHETIC PROTOCOLS IN FOOD-PRODUCING VS. NON-FOOD PRODUCING WILD BOARS

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Wild boars (*Sus scrofa*) are managed and researched on intensively due to their ecological impact. Potent anaesthetic drugs like tiletamine-zolazepam combinations are not allowed in food-producing animals by the EU residue regulation (EU 470/2009). Other combinations are often unsuccessful, which leads to an ethical and legal problem, as well as to potentially dangerous situations for humans. We performed 83 anaesthesias in semi free-ranging female wild boars (6 and 7 years old). All animals were captured in box traps and injected IM behind the ear with a jab stick^a. A) The food-producing animals (n = 21) were pre-medicated with azaperone^b (4.0 mg/kg), plus brotizolam^c (0.004 mg/kg) and induced 15 minutes later IM with ketamine^d (7.0 mg/kg), detomidine^e (0.12 mg/kg), and butorphanol^f (0.15 mg/kg). B) For non-food producing animals (n = 62) we used tiletamine-zolazepam^g (3.0 mg/kg), medetomidine^h (0.06 mg/kg), and butorphanol^f (0.15 mg/kg). All individuals were intubated, received oxygen and isotonic infusions. We measured pedal, palpebral and anal reflexes, heart-rate, respiratory-rate, blood oxygenation, end-tidal CO₂, temperature, arterial blood gases, near-infra-red-spectroscopyⁱ, and electroencephalogram with Sedline¹.

Both protocols induced a surgical state of anaesthesia over 2 hours. Heart-rate was on average 38±6.6 bpm (A) and 54±20 bpm (B), respiratory-rate 25±8 bpm (A) and 35±10 bpm (B) respectively. Body temperature decreased during anaesthesia by 1.1 ± 0.22 °C (A) and 1.7 ± 0.20 °C (B). Females in oestrus required 33% more dosage.

We present a new anaesthesia protocol for food-producing wild boars within the regulations of the EU. The protocol was safe for humans to handle the individuals and showed stable vital parameters.

^aCATS, Daninject, Germany

^bStresnil®, Elanco Animal Health, Bad Homburg, Germany

^cMederantil®, Boehringer Ingelheim-Vetmedica GmbH, Ingelheim, Germany

^dKetamidol®, Richter Pharma AG, Wels, Austria

^eEquidor®, Richter Pharma AG, Wels, Austria

^fAlvegesic®, Alvetra u. Werfft Animal Nutrition GmbH, Vienna, Austria

^gZoletil®100, Virbac, Glattbrugg, Switzerland

^hMedetomidine 20mg/mL, magistral formulation, Vienna, Austria

ⁱMasimo Root 7®, Masimo Österreich GmbH, Vienna, Austria

Ethical statement

Animal experiment permit numbers: 68.205/0224-WF/V/3b/2016 and 68.205/0159-WF/V/3b/2016.

CARDIOVASCULAR EFFECTS OF INTRAMUSCULAR VATINOXAN IN BHARALS (*PSEUDOIS NAYAUR*) IMMOBILISED WITH MEDETOMIDINE-KETAMINE

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Vatinoxan, previously known as MK-467, is an α_2 -adrenoceptor antagonist, that mainly affects the peripheral receptors due to minimal ability to cross the blood-brain barrier. We hypothesised that intramuscular vatinoxan alleviated bradycardia and hypertension in bharals (*Pseudois nayaur*) immobilised with medetomidine-ketamine.

Four male bharals (53–74 kg) were immobilised twice in this randomised clinical crossover study. For one treatment they were darted medetomidine (Zalopine, Orion Corporation, 02200 Espoo, Finland; approximately 80 $\mu\text{g kg}^{-1}$) and ketamine (Ketador, Richter Pharma AG, 4600 Wels, Austria; 1.5 mg kg^{-1}) IM (MK). Oxygen was supplied by a nasal tube when the animals were recumbent. For the other treatment they were additionally administered vatinoxan (V, Vetcare Ltd, 00380 Helsinki, Finland) 25 times the dose of medetomidine IM (MK+V) after collecting the baseline data set: heart rate (HR) and noninvasive mean arterial pressure (MAP). Thereafter data were collected at 15 and 30 minutes. The immobilisation was reversed with IM atipamezole (CP-Pharma HandelsGes. mbH, 31303 Burgsdorf, Germany; five times the dose of medetomidine). Differences between treatments were compared with student's t-test within time-points.

Vatinoxan alleviated the bradycardia and hypertension induced by medetomidine (Table). Immobilisation was sufficient after both treatments, and all recoveries were uneventful.

The improved cardiovascular function induced by vatinoxan may improve anaesthetic safety when immobilising nondomestic ruminants with medetomidine-ketamine.

	baseline	15 minutes	30 minutes
HR MK (min^{-1})	58 \pm 6	52 \pm 7	52 \pm 1
HR MK+V (min^{-1})	55 \pm 18	69 \pm 7*	64 \pm 6*
MAP MK (mmHg)	136 \pm 22	142 \pm 14	130 \pm 17
MAP MK+V (mmHg)	155 \pm 11*	123 \pm 12*	104 \pm 14*

*significantly different from MK ($p < 0.05$).

Ethical approval

Permit number ESAVI/10558/04.10.07/2016, January 17th 2017.

VETERINARY ECOTOXICOLOGY AND ONE HEALTH IN THE ARCTIC

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Exposure to long-range transported industrial chemicals, climate change and diseases is posing a risk to the overall health and populations of Arctic wildlife. Since local communities are relying on the same marine food web as marine mammals in the Arctic, it requires a One Health approach to understand the holistic ecosystem health including that of humans. In the Arctic, exposure to persistent organic pollutants (POPs) is having multiple organ-system effects across taxa, including impacts on neuroendocrine disruption, immune suppression and decreased bone density among others. Modelling shows that exposure to polychlorinated biphenyls (PCBs) may even lead to collapse of half of the killer whale populations while mercury exposure of Greenland inuits have exceeding the guidelines by up to 50 folds. In addition to all this, the warming Arctic climate is suspected to influence abiotic and biotic long-range transport and exposure pathways of contaminants to the Arctic resulting in increases in POP exposure of both wildlife and human populations. Exposure to vector-borne diseases and zoonoses may increase as well through range expansion and introduction of invasive species. It will be important in the future to investigate the effects of these multiple stressors on wildlife and local people to better predict the individual-level health risks. It is within this framework that One Health approaches offer promising opportunities to survey and pinpoint environmental changes that have effects on wildlife and human health.

ANESTHESIA OF FREE-RANGING PERUVIAN FUR SEALS (*ARCTOCEPHALUS AUSTRALIS*) USING MEDETOMIDINE, MIDAZOLAM, AND BUTORPHANOL

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Effective conservation program design relies in part on a comprehensive understanding of population health. As such, over the past decade conservation programs at the Punta San Juan (PSJ) marine protected area in Peru have included projects focusing on the population health of the endangered Peruvian fur seal (PFS; *Arctocephalus australis* unnamed subspecies). Such evaluation of free-ranging pinnipeds requires anesthesia induction in close proximity to water, where suboptimal conditions can result in a partially anesthetized animal drowning. Since 2011, 37 PFS (29 male [111.8 ± 10.5 kg] and 8 female [45.5 ± 7.6 kg]) have been safely anesthetized with a combination of medetomidine^a, midazolam^b, and butorphanol^c (MMB, 0.061 ± 0.019 mg/kg, 0.36 ± 0.12 mg/kg, and 0.36 ± 0.12 mg/kg, respectively) delivered via pistol-fired dart within the PSJ breeding rookery during the peak reproductive period. Supplementation with isoflurane was provided in 28 animals beginning at an average of 22.9 ± 6.5 minutes for an average duration of 33.8 ± 22.7 minutes to facilitate biological sample collection and tagging. Less stimulating activities (e.g. measurements, fur collection, etc.) were possible in most animals without supplementation. Physiologic parameters were closely monitored (HR, RR, temperature, SpO₂, ETCO₂) and spontaneous ventilation was maintained. The majority of animals were intubated and provided with ventilatory support to reduce the incidence of hypercapnia. Atipamezole^d (0.20 ± 0.07 mg/kg), naltrexone^e (0.33 ± 0.12 mg/kg), and flumazenil^f (0.005 ± 0.007 mg/kg) were administered intramuscularly as antagonist drugs 68 ± 20 minutes after darting. Recoveries were smooth and animals displayed coordinated movement on average 9.7 minutes following administration of antagonists.

^aMedetomidine HCl, 20 mg/ml, Wildlife Pharmaceuticals, Inc., Windsor, CO 80550, USA

^bMidazolam HCl, 50 mg/ml, Wildlife Pharmaceuticals, Inc., Windsor, CO 80550, USA

^cButorphanol tartrate, 50 mg/ml, Wildlife Pharmaceuticals, Inc., Windsor, CO 80550, USA

^dAtipamezole HCl, 25 mg/ml, Wildlife Pharmaceuticals, Inc., Windsor, CO 80550, USA

^eNaltrexone HCl, 50 mg/ml, Wildlife Pharmaceuticals, Inc., Windsor, CO 80550, USA

^fFlumazenil, 0.1 mg/ml, West-ward Pharmaceuticals, Eatontown, NJ 07724, USA

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ON A WING AND A PRAYER: INVESTIGATING THE VIRAL SEROPREVALENCE OF A RANGE EXPANDING SPECIES OF FLYING FOX (*PTEROPUS POLIOCEPHALUS*)

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The Grey-headed flying fox (*Pteropus poliocephalus*), one of four mainland species of flying foxes found in Australia, established a permanent camp in the Botanic Park, Adelaide, South Australia in 2011 outside its historic distribution range. This range expansion was likely due to food scarcity within their previous range. The population has increased from ~350 in 2011 to ~20000 today. Between September 2015 and February 2018, sera from approximately 50 bats were collected every six months (301 bats in total over six surveys) and screened for viral seroprevalence (Hendra virus (HeV), coronaviruses and lyssavirus) using a multiplex Luminex fluorescent technology (Luminex, Austin, USA). Seroprevalence across all sessions was 43.2% and 31.6% for HeV and Severe Acute Respiratory syndrome virus (SARS) respectively. HeV seropositivity significantly increased with increased body condition and among seropositive bats. HeV titres were significantly higher in pregnant females and at the second survey (February 2016) but were significantly lower at the fifth survey (August 2017) signifying waxing and waning immunity over this time. Among seropositive bats, SARS titres were significantly lower in non-pregnant females, at the second survey (February 2016) and at the fifth survey (August 2017) again suggesting waxing and waning immunity. All sera were lyssavirus seronegative which provided sufficient evidence to demonstrate freedom from lyssavirus infection in the camp for a design prevalence $\geq 2\%$ and six-month probability of introduction $\leq 5\%$. The results provide evidence that members of the Adelaide camp of Grey-headed flying foxes have been exposed to HeV and SARS virus somewhere along the overlapping distribution range continuum of fruit bats in Australia and South East Asia.

HEALTH ASSESSMENT OF MUSKOX (*OVIPOS MOSCHATUS*) IN NUNAVIK, QUEBEC, CANADA

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Unlike the western Canadian and Alaskan populations that are declining, the population of introduced muskoxen (*Ovibos moschatus moschatus*) in Nunavik, Quebec, Canada seems to be expanding. Data on the health of this population, however, are scarce. Forty-seven muskoxen from two subpopulations were anesthetized [Etorphine (Etorphine 10 mg/ml, Chiron Compounding Pharmacy Inc., Canada) (mean±SD): 40±10 µg/kg and Xylazine (Rompun®, Bayer, Canada): 0.20±0.05 mg/kg] and collared in 2017 as part of a study exploring the relationship between muskox and the declining sympatric migratory caribou (*Rangifer tarandus*) population. There were very few detected abnormalities and the pregnancy rate, assessed by blood pregnancy-associated glycoproteins, was high (94%, n=16). The regular observations of multiple calves per herd were also indicative of a good reproductive success. The serological results suggested the absence of exposure to *Coxiella burnetii* and *Brucella suis*, two zoonotic pathogens that have been suggested to play a role in the decline of muskox populations in western North-America. Antibodies for *Erysipelothrix* sp., an emerging pathogen in this species, were detected in 41% of the animals tested. There was higher seroprevalence for *Besnoitia* sp. (69% vs 37%) and higher prevalence of *Fascioloides magna* shedders (74% vs 42%) in the Hudson Bay subpopulation than in the Ungava Bay subpopulation. These differences suggest higher habitat overlap of the Hudson Bay subpopulation with caribou, that are believed to be the principal reservoir for these parasites. This health evaluation did not document muskox-associated pathogens that could represent a risk for the health of caribou or subsistence hunters.

CHARACTERISATION OF THE ORAL MICROBIOME IN MACROPOD PROGRESSIVE PERIODONTAL DISEASE (LUMPY JAW)-AFFECTED COMPARED WITH HEALTHY MACROPODS USING NEXT-GENERATION SEQUENCING

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Macropod Progressive Periodontal Disease (MPPD; Lumpy Jaw) is the most common cause of morbidity and mortality in captive macropods (MCLELLAND et al., 2019). The pathogenesis of the disease remains incompletely understood. Studies employing culture methods have reported a diverse and inconsistent range of cultivatable bacteria associated with health and disease. Previous studies utilising PCR-denatured gradient gel electrophoresis supported the hypothesis that MPPD is a polymicrobial disease, with no particular organism shown to be necessary or sufficient for disease. We utilised next-generation sequencing to characterise the microbiome of subgingival plaque from captive Macropodidae and Potoroidae at Adelaide and Monarto Zoos, South Australia. Samples were collected opportunistically during clinical investigations and routine health checks. Sequencing reads of sufficient quality were obtained from animals classified as healthy (n = 15), early-stage (gingivitis; n = 4) and advanced (periodontitis ± osteomyelitis; n = 3) MPPD. The phyla Bacteroidetes and Fusobacteria had the highest increase in relative abundance in MPPD; Synergistetes and Spirochaetes were also increased in MPPD. The abundances of genera *Porphyromonas*, *Fusobacterium* and *Bacteroides* increased 3-6 fold in gingivitis cases, and 5-15 fold in advanced cases. Markedly increased abundances of *Aggregatibacter*, *Pyramidobacter*, *Prevotella*, *Peptostreptococcus* and *Desulformicrobium* were also observed. This study is consistent with MPPD being an anaerobe-dominant polymicrobial disease. Bacterial taxa not previously identified in association with MPPD were detected. While the pathogenetic roles of the various taxa documented remain to be elucidated, a better understanding of the microbiology and pathogenesis of MPPD may lead to novel, more effective treatment and preventative strategies.

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CLOUDED LEOPARD (*NEOFELIS NEBULOSA*) MORBIDITY AND MORTALITY IN CAPTIVE POPULATIONS: A COMPREHENSIVE RETROSPECTIVE STUDY OF MEDICAL DATA FROM 271 INDIVIDUALS IN EUROPEAN, ASIAN AND AUSTRALIAN ZOOS

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Medical issues affecting the clouded leopard (*Neofelis nebulosa*) in captivity have not been reviewed so far. The primary objective of this retrospective study was to identify common and significant causes of morbidity and mortality in captive populations of clouded leopards. Clinical and *postmortem* reports were requested from institutions that held clouded leopards from 1934 to 2017.

271 individuals from 44 European, Asian and Australian institutions were included. 344 medical issues from 166 leopards were identified.

The most common causes of death or euthanasia were respiratory issues (17%), maternal neglect (11%), trauma (10%), generalized infectious conditions (9%) and digestive disorders (9%).

The most common causes of morbidity were, by decreasing prevalence, dermatological (21%), digestive (20%), respiratory (16%), musculoskeletal (including trauma - 13%), urinary (10%), cardiovascular (5%) and reproductive (3%) disorders. Neoplastic (7%) and viral (3%) etiologies were notably represented.

Diffuse extensive/self-induced alopecia on the upper back and tail was the most reported disease, with subjective evidence of heritability based on pedigrees analysis. Suspected etiologies included secreting pheochromocytoma-linked chronic arterial hypertension (diffuse telogenization of hair follicles) and specific nerve fiber density (increased sensitivity of affected areas).

(Broncho)pneumonia (7%), nephritis (4%) and enteritis (3%) were particularly prevalent. Pheochromocytoma (1%) was the most common neoplasia, followed by epidermoid carcinoma (<1%), pleural mesothelioma (<1%), and multicentric lymphomas (<1%). Dilated cardiomyopathy (2%) was the most common cardiovascular disorder.

This is the first comprehensive study of the causes of morbidity and mortality in the clouded leopard captive population. Medical and management recommendations have been formulated.

PHARMACOKINETICS AND CLINICAL EFFECTS OF A SINGLE ORAL DOSE OF TRAZODONE IN DOMESTIC GOATS (*CAPRA HIRCUS*) AS A MODEL FOR WILD RUMINANTS

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Trazodone is an anti-anxiety medication commonly used in human and veterinary medicine (JAY et al., 2013; STEVENS et al., 2016). Stress-related trauma is the leading cause of morbidity and mortality in several wild ruminant species. Trazodone could reduce stress in these species and allow for safer capture and handling, thus having a positive effect on animal welfare. The objective of this study was to describe the clinical effects and pharmacokinetic profile of an oral dose of trazodone (Teva Canada Limited, Canada) in domestic goats (*Capra hircus*) as a model for wild ruminants. A pilot study using ethograms and actimetry (Actiwatch 64, CamNtech Ltd, United Kingdom) identified a dose of 10 mg/kg as optimal to reduce activity levels. This dose resulted in a significant increase in time spent sleeping ($P=0.0016$) and lying down ($P=0.008$), and decrease in time spent grooming ($P=0.023$), moving ($P=0.005$), and observing ($P=0.0002$). Activity levels were significantly decreased for 4 hours following administration ($P=0.049$). There were no adverse effects. Time spent eating or ruminating was not affected by trazodone administration ($P>0.05$). The final project evaluated the pharmacokinetics of a single oral dose of 10 mg/kg of trazodone in 7 goats. All animals achieved plasma concentrations over 130 ng/mL that resulted in decreased anxiety in humans and dogs. (JAY et al., 2013; MERCOLINI et al., 2008) Mean elimination half-life was 10.55 ± 6.80 h. All goats achieved C_{max} in 5 to 15 minutes and had detectable plasma concentrations at 24 hours. Trazodone might be useful to decrease stress during management of exotic ruminants. Further research is warranted to establish its efficacy in these species.

Acknowledgements

The authors thank Colombe Otis and Eric Troncy for their guidance with the accelerometers, Guy Beauchamp for statistical assistance, as well as Clément Maincent for technical assistance. This study was supported by the Zoo de Granby Wildlife Health Research Grant. The HPLC-MS/MS analyses were performed on instruments funded by the National Sciences and Engineering Research Council of Canada (F. Beaudry Research Tools and Instruments Grants no. 439748-2013).

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STEM CELL ADMINISTRATION IN CHRONIC RENAL DISEASE IN TWO WILD FELIDS

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Chronic renal disease is commonly reported in geriatric felids. Treatment in these cases is aimed at ensuring quality of life by mitigating disease progression and controlling secondary effects. Autologous stem cells harvested from abdominal subcutaneous fat and administered intravenously has been used to treat renal disease in domestic pets (QUIMBY JM et al 2016). The use of stem cells for treatment of chronic renal disease in two non-domestic felids is presented, applying these protocols followed in domestic small carnivores (QUIMBY JM, BORJESSON DL, 2018).

Case #1: A 15 year old female cheetah (*Acinonyx jubatus*) was diagnosed with chronic renal failure after being presented with acute collapse. Autologous stem cells (33 million) that were harvested from subcutaneous abdominal fat were administered by intraperitoneal infusion twice two weeks apart. Over the course of 7 weeks, and following anesthesia every three or four days for supportive care including intravenous and subcutaneous fluid administration, iron, enalapril, ranitidine, Duphalyte® (Zoetis Spain S.L.), mirtazapine and vitamin B12, the animal recovered normal behavior and appetite, and serum creatinine concentrations decreased from 14.27 mg/dl to 5.58 mg/dl (reference value 0.6-3.7mg/dL). Oral medication (ranitidine, enalapril and mirtazapine) compliance was poor, so standard supportive treatment for chronic renal disease was considered inconsistent. After a month from stem cell administration, the animal died from complications due to aspiration following anesthesia. Necropsy findings included severe bilateral segmental interstitial nephritis with glomerulosclerosis, as well as cranial bronchopneumonia and splenic myelolipoma.

Case #2: An 18 year old female cougar (*Puma concolor*) was diagnosed with chronic renal failure. Conventional oral treatment for felids of mirtazapine, amlodipine, benazepril and omeprazole only lowered serum creatinine concentrations from 6.5 mg/dl to 5.3 mg/dl (reference value 0.6-3.9mg/dL), and the animal remained anorectic and apathetic. Once other potential renal etiologies were eliminated, stem cell administration was provided. Autologous stem cells (30 million) from subcutaneous abdominal fat were administered intravenously under mild sedation using isoflurane as induction and maintenance. This animal received two doses 30 days apart, and oral supportive management was continued. Clinical response was very good with return to normal feeding and activity with fifteen days. Although blood chemistry monitoring was not possible, the animal was considered clinically healthy for three years after the second treatment.

Data for stem cell treatments are currently fragmentary, but these cases suggest a potential for their use in the treatment of chronic renal disease in zoo felids, and becoming a potential tool for improving welfare in geriatric animals. (KINNEY ME, HARMANN R 2019)

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THE NEVER-HEALING HEEL – A FOCUS ON THE AVIAN INTERTARSAL JOINT

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The intertarsal joint is a complex anatomical feature of birds. Located between the tibiotarsus and tarsometatarsus bones, it is an incongruent joint stabilized by many unique structures such as the menisci, collateral ligaments, tibial cartilage, and medial, lateral, extensor and flexor retinacula (MAIERL et al., 2016). To the authors' experience, chronic inflammation of the intertarsal joint is challenging to treat, and choosing the most appropriate therapeutic plan requires precise knowledge of its complex anatomy.

A retrospective case-control study was performed at ZooParc de Beauval, to determine prevalence, identify risk factors, and discuss treatment options and outcome. 35 cases of intertarsal inflammation were identified for the period 01/01/2014 to 31/12/2018. Statistical analysis indicates that Ciconiiformes (prevalence=5.1%), Pelecaniformes (3.1%), and Gruiformes (3.0%) were significantly more affected than other orders ($p<0.01$, $OR=13$). Participation to a free-flight show was a significant risk factor ($p<0.001$, $OR=7.0$). Mean age at onset of clinical signs was 5.7 years. Overall remission rate was 49% and follow-up duration was long (mean = 269 days), associated with poor prognosis in chronic cases.

The tibial cartilage, a fragile fibrocartilaginous structure, was frequently affected (34% cases): chronic inflammation results in compression of the deep flexors that run through it, accounting for typical clinical presentations.

Treatment options included medical management (general NSAIDs, corticoids, tramadol, gabapentin, 94% cases), low-level lasertherapy (49%), joint immobilisation (Robert-Jones bandage, orthosis, 34%), intra-articular corticoid injections (26%), surgical stabilization (17%), physiotherapy (9%), chiropraxis (6%), and intra-articular hyaluronic acid (6%) or PRP (3%) injections. The latter two are promising options to further develop.

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DISTAL TIBIOTARSAL FRACTURE REPAIR IN A SCARLET IBIS (*EUDOCIMUS RUBER*) USING A CIRCULAR EXTERNAL FIXATOR

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Summary

A 4-yr-old adult male Scarlet ibis (*Eudocimus ruber*) was presented to the hospital for non-weight bearing. Physical examination findings and results of diagnostic imaging revealed a closed, comminuted fracture of the distal tibiotarsus with medial displacement of proximal segment and associated soft tissue trauma. The fracture was treated surgically using a circular external fixator as previously described by Kinney et al. in an African sacred ibis (*Threskiornis aethiopicus*) after slight modifications to meet the anatomic requirements (KINNEY et al., 2015). Despite the use of analgesia, the bird was not weight bearing throughout the 32-day-long hospitalization. Radiographs obtained 4 weeks postoperatively revealed bridging callus. The fixator was removed, and the bird started to be weight-bearing 48 hours postoperatively. The bird returned to the show after 4 months of rehabilitation. The use of a circular external fixator may serve as a method of fixation for distal tibiotarsal fractures in other long-legged birds.

Case report

A 4-yr-old male Scarlet ibis (*Eudocimus ruber*) was presented to the ZooParc de Beauval veterinary hospital for non-weight-bearing on the left leg. Physical examination demonstrated crepitus in the tibiotarsal area and radiographs revealed a closed, comminuted fracture of the distal tibiotarsus with medial displacement of the proximal segment and associated soft tissue trauma (Fig. 1). Surgery was scheduled for the following day. The 0.8-kg bird was administered butorphanol (Torbugesic VET 10 mg/ml, Zoetis, 23-25 avenue du Docteur Lannelongue 75014 Paris France; 0.5 mg/kg i.m.), mask induced (5% isoflurane in oxygen), intubated with a "3.5-mm uncuffed endotracheal tube, and maintained on isoflurane (maintenance: 2 - 3 % in oxygen; Iso-Vet 100%; Piramal Healthcare; United Kingdom) for the duration of the procedure. A catheter was placed in the brachial vein and fluid therapy (Lactate Ringer solution; 3 mL/kg/h; Osalia) was maintained throughout the surgery. Surgery site was thoroughly plucked, cleaned and surgically prepared and draped. Closed reduction of the fracture was accomplished. Using small stab incisions, six 1-mm pins were driven through both corticals, from caudal to cranial and lateral to medial of the tibiotarsus, using a handheld chuck - two in the distal segment and four in the proximal segment. The circular external skeletal fixator (ESF) consisted of 3 parallel ring fixators made of plastic infusion tubing fixed to the transverse pins and anchored firmly in position with polymethyl-methacrylate (Technovit 6091, Kulzer, Heraeus Kulzer GmbH, Philipp-Reis-Strasse 8/13 - 61273 Wehrheim, Allemagne). The ring fixators were fixed together using 3 connecting bars (2-mm pins) inserted perpendicularly into them (Fig. 2). A thin layer of bandage material (Vetrap, 3M Animal Care Products, St. Paul, Minnesota 55141, USA) was placed over the fixator before recovery. Postoperative radiographs revealed satisfactory placement of the pins and showed reasonable alignment (Fig. 3). Anaesthetic recovery was smooth and uneventful. Treatment follow-up included meloxicam (Metacam 1.5 mg/ml, Boehringer Ingelheim Vetmedica GmbH, 55216 Ingelheim/Rhein, Allemagne, 1 mg/kg p.o. s.i.d. for 5 days), tramadol (Tramadol LP

Sandoz 100mg, Novartis, Basel, Switzerland, 10 mg/kg p.o. b.i.d. for 30 days) and gabapentine (Gabapentine Sandoz 100mg, Novartis, Basel, Switzerland, 11 mg/kg p.o. b.i.d. for 30 days). Force-feeding was also needed once as the bird presented anorexia for 4 days after the surgery. Despite analgesia, the bird never used its leg until fixator removal. Brief anaesthesia (Iso-Vet 100%; Piramal Healthcare; United Kingdom) were performed the following 2 weeks every 3 days for local care of the fixators and lasertherapy (Programme "Exotics; musculoskeletal"; 120J; Cube 3 K-Laser, Mikan). *Dorsoplantar (DPI) and lateral* radiographs were obtained at 4, 8, 12, and 16 weeks postoperatively (Fig. 4). At 4 weeks, the external fixator was removed after radiographic evidence of fracture alignment, bridging callus and palpable stability of the fracture site. After external fixator removal, the bird progressively put more weight on the left leg, and after further 15 days, no lameness was observed anymore. At 8 weeks post-surgery, the bird was moved to a larger enclosure and at 12 weeks post-surgery, the bird was finally able to be replaced in the group.

Discussion

Tibiotarsal fractures are among the more common orthopaedic problems encountered in birds (KAVANAGH, 1997). Consideration of some special features of avian bone (thin and brittle cortical, endosteal blood supply and endosteal callus formation) is particularly important to ensure good fracture healing (KAVANAGH, 1997). Multiples techniques for repairing bone fractures in birds have been described. Initial stabilization with a splint can be attempted in small birds but may be insufficient for long-term management because of the lack of rigid fixation and the need for frequent bandage changes (KINNEY et al., 2015).

Intramedullary pinning has been well described in various bird species, but common complications of traditional fixation devices include fracture instability, implant migration, implant rotation, joint penetration, intramedullary blood supply destruction and absence of endosteal callus formation (STEJSKAL et al., 2011).

Most tibiotarsal fractures are successfully managed by surgical reduction and stabilization with a TIF (External skeletal fixator intramedullary pin tie-in), as this combines the installation of an intramedullary pin and external fixation using external pins to prevent implant rotation and fracture instability. However, a routine TIF normally fixes the fracture at one of two sides laterally, but not all around as the used circular external fixator, which may be necessary depending on the fracture.

In general other comorbidities (eg, systemic infections and visual deficits) can negatively affect the rehabilitation of birds and can sometimes result in euthanasia (BUENO et al., 2015).

Other methods include the use of interlocking nails (IN) and titanium microplates. The use of IN consists of an intramedullary (IM) pin fixed by perpendicular screws inserted in nails. It is particularly indicated in the treatment of comminuted, middiaphysis fracture (HOLLAMBY et al., 2004). The successful use of IN in a wild, adult bald eagle (*Haliaeetus leucocephalus*) with an open, grade II, transverse, midshaft, Winquist–Hansen type-II–comminuted left tibiotarsal fracture has shown efficacy but the cost of the system and the experience required to appropriately use it are inconvenient (HOLLAMBY et al., 2004).

Plates may be another option. A locking compression plate is placed externally to the skin surface and screws are placed in the proximal and distal fracture segment engaging all cis and trans cortices. However, the thin bone corticals give poor screw grip and a more extensive surgical procedure is required (GOUVEA et al., 2011).

Fractures of the tibiotarsus provide fixation challenges because of the relatively small medullary cavity in some birds. In addition, the distal fracture location in this case added challenges. The external skeletal fixator was ideal in this situation as it addresses all the destabilising forces, especially in 3 dimensions contrary to TIF, preserves the intramedullary blood supply contrary to IM and IN techniques, and avoids soft tissue damage close to the fracture site, as previously described for

tarsometatarsal fracture repair of an African bald ibis (*Threskiornis aethiopicus*) (KINNEY et al., 2015). Early limb use is possible and the incidence of fracture disease is reduced. It is cheap and relatively quick to apply and remove. The use of transverse pins as connecting rods joined by infusion tubings and connecting bars enable to assess the adequacy of the fracture reduction and limb alignment and permits suitable adjustments before committing to the rigidity of cured acrylic cement (KINNEY et al., 2015). The use of a circular external fixator should be considered in long-legged birds with distal tibiotarsal fractures and small distal fracture segments that may preclude the use of more commonly used stabilization techniques.



Fig. 1: Dorsoplantar (DPI) (a) and lateral (b) radiograph demonstrating a comminuted fracture of the distal left (L) tibiotarsus in a Scarlet ibis.



Fig. 2: Circular external fixator in a Scarlet ibis.

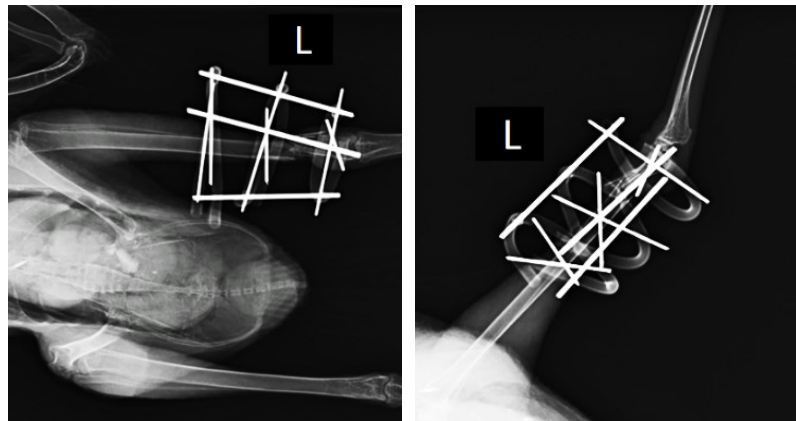


Fig. 3: Dorsoplantar (DPI) (a) and lateral (b) radiograph after placement of a circular external fixator in a Scarlet ibis.

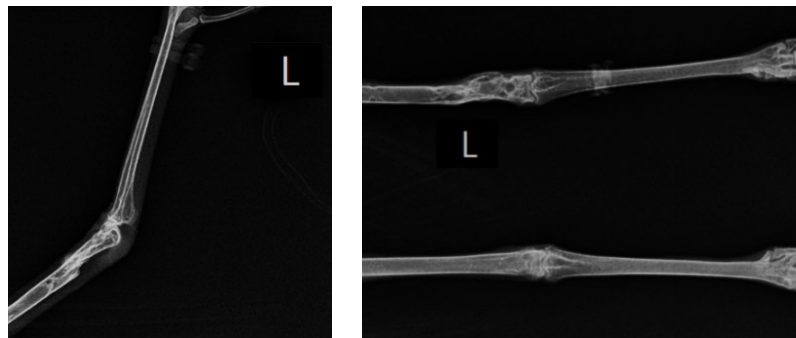


Fig. 4: Dorsoplantar (DPI) (a) and lateral (b) radiograph 2 months after removal of a circular external fixator in a Scarlet ibis.

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TREATMENT OF EXUBERANT GRANULATION TISSUE IN AN UMBRELLA COCKATOO (*CACATUA ALBA*) WITH STRONTIUM RADIATION

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A 26-year-old female umbrella cockatoo (*Cacatua alba*) presented for reoccurrence of a soft tissue mass extending from a crack on the rhinotheca. The mass was originally observed 12 years' prior following unknown trauma. Histopathology after initial removal was reported as fibroblastic and collagen proliferation with secondary heterophilic inflammation, mucinous degeneration, and focal extensive epidermal ulceration, consistent with inflammatory granulation tissue. The mass reoccurred three additional times despite electrosurgical removal and cryogenic surgery. Biopsy samples of the tissue mass were submitted following the subsequent procedures with similar interpretations as the first. On the fourth surgical resection, strontium-90 radiotherapy was applied to the site immediately following the surgical procedure. There is a lack of information regarding the presence of exuberant granulation tissue originating from the epithelium following trauma in avian species. There are numerous reports of this fibroproliferative disorder in horses with wounds on the distal limbs (WISE et al., 2006, VARASABI et al., 2018). Treatment has typically included chemical cautery, cryogenic surgery, and surgical reduction, but reoccurrence is common. New experimental treatments have been published, including administration of topical of virus-derived proteins (Wise et al., 2006), injection of formaldehyde (VARASABI et al., 2018), and application of acupuncture beads (FRAUENFELDER 2008), among many others. This report describes the successful removal inflammatory granulation tissue and concurrent novel use of radiation therapy to successfully treat recurrent inflammatory granulation tissue in an umbrella cockatoo. Approximately 2 years post radiation there is no sign of the tissue mass on the rhinotheca along with significant beak remodeling.

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VETERINARY MANAGEMENT OF HIGH PROFILE MARINE MAMMAL SPECIES

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At first glance and in contrast to more familiar terrestrial mammals applying modern zoo animal medicine to marine mammals, especially cetaceans, looks very challenging. Cetaceans are fully adapted to life in water and getting hands-on with them appears to require getting them out of the water. Their respiratory physiology is different, their diet as obligate piscivores is unusual, their environment needs careful management, and their hairless skin appears delicate. Pinnipeds are perhaps more familiar, but some of the same caveats apply. At the same time, they are susceptible to a similar of diseases which may need preventative and curative care. The keeping of cetaceans under human care dates back to the 1930s, with their popularity increasing rapidly with better understanding of their needs through the 1960s to the present day. Most marine mammals under human care are trained, either to do educational presentations, for research, or even for military purposes, and it is this training which is the key to their care. Intense co-operation between trainers and veterinarians over the past 30 years has allowed us to do procedures which are impossible in most zoo mammals without anaesthesia, such as weekly weighing, blood sampling, ultrasound, gastric, faecal and urine sampling, gastroscopies and even voluntary bronchoscopies. This presentation will review the more common diseases and disorders of cetaceans and other marine mammals and their management, with emphasis on the ways in which the obvious difficulties can be overcome.

NUTRITIONAL CONCERNS OF REHABILITATED AND LONG TERM CAPTIVE WILD FLORIDA MANATEES (*TRICHECHUS MANATUS LATIROSTRIS*) WITH REFERENCE TO HEALTHY WILD POPULATIONS

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Florida manatees (*Trichechus manatus latirostris*) in rehabilitation centers and zoological facilities are typically fed commercially grown lettuces. Compared to natural seagrasses, commercially grown lettuces contain 2-6 times greater caloric density and have higher digestibility with lower fiber content. Prolonged feeding of commercial lettuces has been linked to diabetes mellitus and may negatively impact survivability. Diabetes was diagnosed based on hyperglycemia, glycosuria and elevated fructosamine in a rehabilitated manatee that was released and did not survive other clinical cases are suspected with elevated fructosamine and high body mass index (BMI). Several manatees in a long-term captive population died with clinical signs consistent with unregulated diabetes including cardiovascular and renal disease. Pancreatic islet fibrosis (PIF), similar to that in rock hyrax, has also been described in a long term captive manatee. Fructosamine elevations reflect persistent hyperglycemia and are strong indicators of Type 2 diabetes in other mammals. Fructosamine reference values from wild healthy Florida manatees are available (range 149-317 $\mu\text{mol/L}$, $n=25$). Evaluation of long term captive manatees revealed elevated fructosamine levels compared to these values (range 1000-388 $\mu\text{mol/L}$; $n=7$ samples from 4 animals), supportive of a diabetic state. Changing the diet to ~90% grass hay successfully reduced fructosamine values in two of these manatees were complete follow up is available. Blood vitamin levels in animals on commercial lettuce diets may also vary from free-ranging animals. Alpha-tocopherol values for wild manatees (mean 0.95 $\mu\text{g/ml}$, $n=25$) are lower than selected long term captive (LTC, mean 1.51 $\mu\text{g/ml}$, $n=4$;) and some rehabilitating manatees (RM, mean 1.15 $\mu\text{g/ml}$, $n=9$) (LTC). 25-hydroxyvitamin D levels (LTC mean 92.0 pmol/dl , $n=4$; RM mean 86.3 pmol/dl , $n=9$) were actually above the mean for wild manatees (mean 14.56 pmol/dl , $n=25$) with several individuals 10X over. Digestibility varies between grass hay and commercial lettuce diets, with grass hay appearing to better mimic seagrass diets. Manatees fed grass hay had notable changes in the fecal production of volatile fatty acids (VFA) compared to manatees fed romaine exclusively with an increase in the total amount of VFA's and a shift from approximately equal amounts of acetic and propionic acids to a relative concentration of acetic>propionic>butyric acid. In wild aquatic hindgut fermenters, manatees and sea turtles, the relative concentration acetic>butyric>propionic acid. Moving to a captive diet higher in hays, or other sources of fiber, may better mimic a seagrass diet and improve a variety of health parameters in the Florida manatee.

PREPARING A POPULATION OF BOTTLENOSE DOLPHINS (*TURSIOPS TRUNCATUS*) TO MOVE TO A SANCTUARY SETTING

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This talk will review the process and frameworks National Aquarium staff have utilized to guide preparing a population of bottlenose dolphins (*Tursiops truncatus*) to move from their current home to a new, outdoor environment. Our vision is to ensure that each dolphin thrives through every aspect of the training, transportation, and acclimation phases.

The population is currently housed in an indoor facility with artificial, recirculating seawater. There are seven (2.5) dolphins ranging in age from 10 – 26 years. Six (2.5) dolphins were born on-site and have never experienced any other housing situation. One animal was born in an outdoor, artificial seawater facility. A few major risk factors for health and welfare identified for moving to an outdoor, natural seawater environment included lack of experience in transportation or acclimation, exposure to novel microbiomes, and ingestion of foreign objects in animal with limited exposure to natural objects.

This talk will focus specifically on two frameworks used to guide training and site selection efforts.

1) Transition Plan: Overall structure for pre-transportation, transportation, and acclimation phases that guides short- and long-term training, research, and facility goals.

2) Individual Dolphin Assessment Matrix: Guides development of specific training and resiliency goals for each dolphin and indicates which animals are best prepared for the move.

We feel aspects of these frameworks will be of interest to those working with other species, as well as those working bottlenose dolphins.

PATHOLOGICAL FINDINGS IN NORTH SEA AND BALTIC GREY SEAL AND HARBOUR SEAL INTESTINES ASSOCIATED WITH ACANTHOCEPHALAN INFECTIONS

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Grey seals (*Halichoerus grypus*) and harbour seals (*Phoca vitulina*) are common North Sea and Baltic seal species and final hosts of several parasite species. Acanthocephalan infections are increasingly observed. *Corynosoma strumosum/magdalenae* infect both seal species' small intestines. Baltic grey seals display colonic *Corynosoma semerme* infections associated with ulcers and thickened colonic walls as part of the Baltic Seal Disease Complex (BSDC). The lesions' pathogenesis is still unknown.

This study focuses on lesion differences and parasite-lesion correlation. Infected Baltic grey seal (n=65) and North Sea harbour seal (n=504) intestines collected in Germany, Poland and Estonia from 1998-2017 were investigated macroscopically and histologically. Male grey seals predominated (46 males/19 females), while the harbour seal sex ratio was almost equal (265 males/239 females).

Both species' small intestines were mildly-moderately infected, whereas grey seals showed severe colonic infections not found in harbour seals. Levels of infection were determined semiquantitatively. Harbour seals mainly displayed a chronic granulomatous eosinophilic mural enteritis (70.0 %). Grey seals predominantly showed a chronic eosinophilic lymphoplasmacytic erosive-ulcerative colitis (36.9 %) and tunica muscularis hypertrophy (16.9 %). In contrast to harbour seals, the grey seals' lesions were visible macroscopically. Acanthocephalan infections significantly enhance the probability to 60 % both for enteritis in harbour seals and colitis in grey seals.

Harbour and grey seals differ in acanthocephalan distribution and lesions' extent, especially in the colon. Findings indicate still prevailing BSDC symptoms. Acanthocephalans and associated lesions may be suitable seal health indicators and the colon a target organ for Baltic grey seal health investigations.

MANAGEMENT AND HUSBANDRY CONSIDERATIONS FOR STRANDED CETACEANS

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Rescue, rehabilitation and disposition of stranded cetaceans has undergone a substantial evolution in the approach and the decision-making process in the last decades. On site therapy and management choices are heavily impacted by the experience of the veterinarian, government agencies involved and the primary responders. Utilization of medications during initial response on the beach (e.g., calcium 20 mg/kg IM, vitamin E -selenium 0.06mg/kg IM, steroids, oxygen supplementation) may improve transport success and alleviate secondary complications including myositis. The use of calcium in particular may be an important component to successful cetacean rescue and rehabilitation since cetaceans normally ingest large amounts of calcium with a whole fish diet. If deficient in nutritional intake prior to a stranding, inadequate intake after rescue, and the use of acid reducing medications they are more prone to hypocalcemia which can manifest with myositis, cramping and scoliosis 2 to 5 days post event. Post rescue therapy should include follow-up medication with additional oral vitamin E and calcium as part of a nutritional plan for a number of weeks. Additional medical management includes diagnosing, treating and preventing, pneumonia, ulcerative or infectious gastritis and enterocolitis. Rehabilitation facility characteristics should allow animals to swim in straight lines to alleviate and prevent muscle contraction and subsequent scoliosis. It is recommended that minimal pool dimensions for a dolphin be 12 by 18 meters. Auditory evoked potential testing to evaluate hearing should always be part of the health evaluation, as animals with hearing loss should not be released. Additionally, neonate-juvenile animals are considered incapable of adapting back to the wild and should not be released. A more recent improvement to holistic care for stranded individuals is to utilize human caretakers as active cohort or parenteral substitutes during the rehabilitation period. Cetaceans are highly social, and all ages respond to increased interaction. Younger animals adapt positively to more interactive time (14 to 24 hours per day) during the initial recovery and through the isolation period until appropriate cohorts are available. Satellite tagging of released animals is essential to better document post stranding survivability to guide management choices for individual animals in the future. It also provides unique opportunities for capturing species-level data, such as location of home territories, feeding strategies, and migration routes that may contribute to future environmental protection.

WILDLIFE EMERGING INFECTIOUS DISEASES: RISKS FOR A SEMI-URBAN ZOO, IMPORTANCE OF SURVEILLANCE PROGRAM, AND OPPORTUNITIES FOR RESEARCH

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The risk of disease transmission from native wildlife to animals maintained in zoological institutions has been recognized for many years. Emerging infectious diseases originating from wildlife are increasing. Surveillance is the key to early detection and prevention. Over the past decade, four major emerging diseases were first identified at the Toronto Zoo. Aquatic bird bornavirus 1 was identified as a cause of neurological disease and mortality in free-ranging Canada geese (*Branta canadensis*) and trumpeter swans (*Cygnus buccinator*) on the zoo site in 2011. This virus was also responsible for the death of a captive emu (*Dromaius novaehollandiae*). An outbreak of haemolytic anaemia in several reindeer (*Rangifer tarandus*) and American elk (*Cervus canadensis*) at the zoo in 2012 led to the first recognition of cervid babesiosis in Ontario. *Batrachochytrium dendrobatidis* was identified in a Puerto Rican crested toad (*Peltophryne lemur*) for the first time in 2018. This captive toad was most likely infected by a free-roaming American toad (*Anaxyrus americanus*) that had been inadvertently introduced into the same Zoo pavilion. *Ophidiomyces ophiodiicola* (snake fungal disease) was isolated from an Eastern milksnake (*Lampropeltis triangulum Triangulum*) in 2019. This wild snake was found inside a greenhouse of the zoo. This highlights the role of zoos as critical part of wildlife disease surveillance programs. Subsequent to the detection of these agents on zoo site, more extensive surveys have been completed.

IDENTIFICATION OF AVIAN HAEMOSPORIDIAN PARASITES IN TISSUE SAMPLES BY CHROMOGENIC IN SITU HYBRIDIZATION

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Avian haemosporidian parasites can cause severe disease in their hosts and mortalities have been associated with excessive exo-erythrocytic merogony of *Plasmodium* parasites and the development of megalomeronts by parasites of the genera *Haemoproteus* and *Leucocytozoon*. Post-mortem diagnosis of haemosporidiosis is currently accomplished by PCR or identification of parasite stages in histological sections. However, sequences are sometimes not reliably obtained and the detection of exoerythrocytic stages in histological sections is time-consuming and difficult, because parasites are easily overlooked. More importantly, the genus- or even species discrimination of parasite tissue stages based on morphological characters is challenging. Here, we present chromogenic in situ hybridization (CISH) as a tool for the detection and identification of exoerythrocytic stages of avian haemosporidian parasites in histological sections from formalin-fixed paraffin-embedded tissues. Molecular probes were designed to target the 18S ribosomal RNA of parasites. The probes were tested on tissue sections from samples confirmed PCR-positive for different species of the genera *Plasmodium*, *Haemoproteus* and *Leucocytozoon*. CISH showed robust positive signals in different organs of tested samples. Specificity of the signal was validated by the presence of parasites in corresponding HE-stained sections. Signals were observed within red blood cells, corresponding to erythrocytic stages, and in tissues, correlating with exoerythrocytic stages (meronts and megalomeronts). The results indicate that exoerythrocytic stages of haemosporidian parasites can be easily detected and discriminated in tissue sections by CISH. Thus, CISH proves to be a powerful method for the unequivocal diagnosis of haemosporidiosis in histopathologic material.

REOCCURRENCE OF *ANGIOSTRONGYLUS DUJARDINI* IN CALLITRICHIDAE AFTER A CHANGE IN THERAPEUTIC MANAGEMENT – EPIDEMIOLOGY, DIAGNOSTIC METHODS AND A NEW PROPHYLACTIC APPROACH

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Angiostrongylus dujardini is a metastrongylid nematode infecting rodents as final hosts and snails as intermediate hosts. Accidental final hosts like callitrichids are infected after consumption of infected snails. Their larvae develop into adults in the heart and lungs and can cause pneumonia and possibly death.

In 2002 two golden-headed lion tamarins (*Leontopithecus chrysomelas*) and in 2009 one emperor tamarin (*Saguinus imperator*) were lost due to *A. dujardini* infection. Larvae were also detected in mice and snails proving, that the parasite life cycle was completed causing permanent threat to the cage inhabitants. Therefore, treatment with ivermectin (0,2mg/kg per os every two weeks) was applied. After eight years without casualties, treatment was prolonged to a single administration monthly to reduce the metabolic burden for the animals. In summer 2018 two juvenile *L. chrysomelas* suddenly died and *A. dujardini* was diagnosed again during necropsy, proven via PCR. Additionally, nematode stages and antigen were also detected in snails (*Limax maximus*) and mice (*Mus musculus*, *Apodemus sylvaticus*) collected in the department. As expected cockroaches (*Periplaneta australasiae*) tested negative. The results show that the life cycle was either still complete even after several years or *A. dujardini* had been reintroduced by mice. Treatment management was changed to another, modern macrocyclic lactone moxidectin (0,2mg/kg per os once per month) which in dogs and cats accumulates in fatty tissue and reaches a protective steady state after approximately four monthly spot-on treatments. So far, no further incidents occurred and all animal sera tested negative for *A. dujardini* antigen.

CLINICAL PRESENTATION OF PROVENTRICULAR NEMATODIASIS DUE TO *SYNHIMANTUS NASUTA* IN LORIKEETS (*TRICHOGLOSSUS* SPP.)

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Background: In July 2018, *Synhimantus nasuta* infections in a captive flock of 84 lorikeets (*Trichoglossus* spp.) were responsible for clinical illnesses and two fatalities. This spirurid induces a proliferative proventriculitis and anaemia due to gastric blood loss. Definitive diagnosis is complicated by intermittent shedding of the ova.

Objective: To investigate the pertinence of adjunct physical and clinicopathological findings to determine probable cases of *Synhimantus nasuta* infection in absence of ova shedding in an outbreak situation.

Methods: In 11 lorikeets suspected to be infected based on at least an abnormal finding on their physical examination (lethargy, feather damaging behaviour on the ventrum, weight loss, pale iris), the presence of 5 additional parameters were documented: anaemia, eosinophilia, proventricular diameter-to-keel height ratio (PKR)>0.48, proventricular barium filling defect, and positive fecal occult blood test. A total score (x/9) was calculated by combining all findings. *Synhimantus nasuta* infection was confirmed in 4 of these individuals by Wisconsin faecal exam.

Results: Birds with confirmed infections (n=4/11) presented with both low (2-3/9, n=2/4) or high (7/9, n=2/4) total scores. Individuals with high scores presented iris pallor, which was concurrent with anaemia. Fecal occult blood was present in all confirmed cases and 4/11 of suspect cases. PKR>0.48 was highly prevalent and only observed in birds with active shedding (3/4).

Conclusion: During a *Synhimantus nasuta* eclosion, physical examination may help identify suspect cases, including individuals requiring immediate medical attention. In the absence of ova shedding, PKV>0.48 and detection of occult faecal blood suggest a probable infection.

ANTIBODY RESPONSE TO EPSILON TOXIN OF *CLOSTRIDIUM PERFRINGENS* IN CAPTIVE ADULT SPRINGBOK (*ANTIDORCAS MARSUPIALIS*), IMPALA (*AEPYCEROS MELAMPUS*), ALPACA (*VICUGNA PACOS*) AND BENNETT'S WALLABY (*MACROPUS RUFOGRISEUS*) OVER A YEAR

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Enterotoxemia is an important issue in various zoological taxa. In this study, serologic responses over a year period after vaccination with a multivalent clostridial vaccine (Miloxan® Boehringer Ingelheim – Merial, Lyon, France) were evaluated in 10 adult springboks (*Antidorcas marsupialis*), twelve impalas (*Aepyceros melampus*), seven alpacas (*Vicugna pacos*) and five Bennett's wallabies (*Macropus rufogriseus*). Antibody production to the *Clostridium perfringens* type D epsilon toxin component of the vaccine was measured using an indirect enzyme-linked immunosorbent assay and determined as the percentage of inhibition (% inhib). Initial % inhib was [0.01 - 18.9]%. All animals received initial vaccination with a booster vaccine four weeks apart. Serum samples were collected at days 0 (non-vaccinated), 15, 30, 60, 180 and 360 post-vaccination (pv) for analysis. The vaccine induced a high antibody response that peaked at day 15, 30, 60 pv in springboks ($p < 0.01$), day 30 and 60 pv in impalas ($p < 0.01$) and at day 60 pv for the other species ($p < 0.01$). The booster vaccine was followed by a high humoral response. The antibody response then slowly decreased with time. The antibody response was significantly higher at day 360 pv than at day 0 in wallabies and alpacas ($p < 0.01$). In impala and springbok, it appeared that a booster every 6 months might be required to maintain an antibody response above baseline ($p < 0.01$). Since no challenge studies were performed, it is unknown whether the measured humoral immune responses would have been protective. Further research is warranted to investigate antibody's protective effects to inoculation challenge in nondomestic species.

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IDENTIFYING LEPROSY IN EURASIAN RED SQUIRRELS (*SCIURUS VULGARIS*): LESSONS LEARNED IN TWO BRITISH POPULATIONS

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The discovery of leprosy in Eurasian red squirrels (*Sciurus vulgaris*) caused by *Mycobacterium leprae* and *Mycobacterium lepromatosis*, and the original description of clinical signs was based on findings in carcasses obtained as part of an ongoing disease surveillance programme in Scotland. Further information came from carcasses within the British Isles submitted following these initial descriptions. We studied two British island squirrel populations in which the presence of leprosy had been previously confirmed over 2 years. We assessed the clinical spectrum of disease in live squirrels and optimised diagnostic approaches for use in live animals.

In 188 trap/ sample events we assessed 125 squirrels, 73 from Brownsea Island, England and 52 from the Isle of Arran, Scotland. While we did not see clinical cases of leprosy on Arran and were only able to detect *M. leprae* DNA in two animals there, the Brownsea population allowed us to document a wide spectrum of lesions caused by *M. leprae*, and their progression over time. Unexpectedly, we did not detect *M. lepromatosis* DNA in any squirrels. A combination of clinical assessment using a defined scoring scheme, anti-PGL-I lateral flow assay and mycobacterial DNA isolation from punch-biopsies was used to diagnose leprosy in squirrels and improved our ability to identify squirrel leprosy cases.

This information will assist in identification of cases of leprosy where suspicious skin lesions are observed, and in determining whether squirrel leprosy is limited to the British Isles or is occurring throughout the species' range.

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DIAGNOSTICS AND TREATMENT OF EEHV-1B IN A 2-YEAR-OLD ASIAN ELEPHANT (*ELEPHAS MAXIMUS*) CALF AT ARTIS AMSTERDAM ROYAL ZOO

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Elephant endotheliotropic herpesvirus can cause an acute, often fatal hemorrhagic disease (EEHV-HD) in elephants (HOWARD et al., 2018). A 2-year-old female Asian elephant (*Elephas maximus*) was presented with petechial hemorrhages on her tongue. Oral famciclovir (15 mg/kg, Famciclovir Sandoz®, Sandoz BV, the Netherlands) was given immediately. The dam was sedated prior to sedating the calf. Petechial hemorrhages were also observed on the vulvar mucosa. EDTA samples were taken and submitted to the Animal and Plant Health Agency, Addlestone, UK, for EEHV PCR and qPCR. Rectal fluids and intramuscular trimethoprim/sulfamethoxazol (Diatrim24%, Dechra Veterinary Products, the Netherlands) were administered. qPCR was positive for EEHV-1B at 2.08×10^6 vge/ml. Cross matches revealed compatibility of three donor elephants with the recipient. The following three days the cow and calf were sedated for diagnostic sampling and administration of intravenous plasma infusions, rectal famciclovir and trimethoprim/sulfamethoxazole to the calf. qPCR's and manual count of the white blood cells and platelets were done each time. On Day 4 the viral load had dropped substantially. On Day 8 both elephants were sedated again. The petechial hemorrhages on the tongue had disappeared. Viral load had slightly increased, but WBC and platelets had increased to $30.3 \times 10^9/L$ and $1256 \times 10^9/L$ respectively, indicating a strong immune response. It was decided to cease treatment. This case may be illustrative for a normal primary EEHV-1B infection in a calf and furthers the knowledge of the pathophysiology of EEHV-1B. The importance of manually monitoring monocytes and platelets, teamwork, collaboration between institutions, animal access, daily health checks and EEHV preparedness are highlighted.

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DIAPHRAGMATIC PERITONEAL PERICARDIAL HERNIA IN A EURASIAN LYNX (*LYNX LYNX*)

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Introduction

Congenital malformations of diaphragm are rarely described in domestic animals. The diaphragmatic peritoneal pericardial hernia DPPH is the structural congenital defect most commonly found in dogs and cats^[1,2]. The peritoneal and pericardial cavities remain connected and abdominal content is often found within the pericardial sac^[1,3].

Clinical signs are non-specific and among others, gastrointestinal, cardiac, respiratory or neurological problems may appear^[1,3]. Some animals do not show any clinical signs and DPPH is only found incidentally or during necropsy.

DPPH diagnosis in wild animals is anecdotic^[4] therefore the understanding of this congenital malformation in wildlife is also limited.

Material and methods

This work describes the clinical case of a 9-year-old female Bobcat (*Lynx lynx*) from Vigozoo, which presented clinical signs of respiratory distress and was incidentally diagnosed with a diaphragmatic peritoneal pericardial hernia (DPPH) in December 2017.

The animal was anaesthetised for treatment of an otolithoma and suffered important respiratory distress during the examination. A radiographic study showed an enlarged rounded cardiac shadow with intestinal loops inside the pericardium and dorsal relocations of trachea. The animal had never shown any clinical signs previously. Blood work revealed moderate neutropenia, mild azotemia and mild increase of hepatic enzymes.

The animal was given 40µg/kg medetomidine (Domtor® 1mg/ml, Esteve S.A, Barcelona, Spain), 1.5mg/kg ketamine (Ketamidor® 100mg/ml, Richter Pharma Ag, Wells, Austria) and 0.15 mg/kg butorphanol (Butomidor® 10mg/ml, Richter Pharma Ag, Wells, Austria) to be transferred to the Rof Codina Veterinary Hospital. Once there, the same protocol was used to immobilize the animal and 1mg/kg propofol (Propovet® 10mg, Abbot, Breda, Nederland) was given intravenously for endotracheal intubation. 1.5-2.4% Sevoflurane (Sevoflo®, Abbot, Breda, Nederland) was used for anaesthetic maintenance. 2mg/kg Furosemide (Seguril® 20mg/2ml, Sanofi-aventis S.A., Barcelona, Spain) was administered intravenously to avoid pulmonary oedema, and cisatracurium (Cisatracurio Normon® 2 mg/ml, Laboratorios Normon, Madrid, Spain) was given 0,1mg/kg IV to assist mechanical ventilation.

As antibiotic prophylaxis, cephazolin (Cefazolina Normon® 1gr, Laboratorios Normon, Madrid, Spain) was given at a dose of 22mg/kg and IV and for analgesia, 0,3mg/kg/IV methadone (Metasedin® 10mg/ml, Laboratorios Esteve S.A, Barcelona, Spain) was administered before surgical procedure.

Lactates Ringer's (B. Braun Medical, SA, Barcelona, Spain) was administered at a 5 ml/kg/h infusion rate. The animal was positioned in supine decubitus and antitrendelemburg position. The surgery was initiated with midline abdominal cranial incision. A ventral diaphragmatic defect was found directly connecting the abdominal cavity with the pericardium with hepatic lobules, pancreas and most of the small intestine present therein. The herniated organs were manually reduced with no complication as adhesions was not observed.

After repositioning, expansion of pulmonary lobules was gradually conducted by increasing small inspiratory pressure and PEEP application was used in order to improve alveolar recruitment, reduce presence of atelectasis, avoid barotrauma and decrease the risk of pulmonary oedema.

The animal was stabilized and a thoracic drain was placed to re-establish negative pressure, whilst the diaphragmatic hernia was closed with non-absorbable monofilament (nylon 0/0) suture material. A radiographic study was completed that confirmed the absence of pneumothorax during pulmonary expansion.

Once adequate breathing was observed, the thoracic drain was removed.

Discussion

When the diagnosis is reached incidentally in adulthood, both a surgical and conservative treatment should be considered ^[3]. On the one hand, some studies have shown that there is no significant difference in survival times for the treatments indicated ^[2, 5]. On the other hand, if the treatment chosen is conservative, clinical signs may worsen and may even lead to the death of the animal ^[3].

A quick surgical action is related to a decrease of adherence and a lower risk of pulmonary oedema ^[3]. The possible relationship between incarcerated hepatic tissue in DPPH and the development of cysts and hepatic neoplasm in the feline species has been documented in recent years ^[6, 7]. In our case, the animal had not shown clinical symptoms till date, but suffered a rapid and severe decomposition that resulted in severe respiratory failure with acute pulmonary oedema, which could likely be attributed to external factors such as management stress or the administration of some anaesthetic drugs.

As a general rule, the decision for establishing a treatment should be made based on the presence or absence of previous clinical signs ^[2, 5]. In our case surgical treatment was considered the best therapeutic option considering the difficulties for treatment in a wild animal and the serious clinical symptoms.

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INFLUENCE OF EXPOSURE TO GLYPHOSATE AND ATRAZINE ON SKELETAL DEVELOPMENT IN *PODOCNEMIS UNIFILIS* (TESTUDINES: PODOCNEMIDIDAE)

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A third of European reptile species are at high risk of exposure to pesticides and the same is likely true in Brazil, which has been leading in the use of agrochemicals. This study aimed to investigate skeletal changes in Terekay embryos (*Podocnemis unifilis*) submitted to isolated or combined egg's exposure to glyphosate and/or atrazine (Animal Ethics Committee CEUA/UFU, Protocol nº55/12). Forty-two eggs were divided into four groups: Atrazine (A) in concentration of 2 µg.L⁻¹ (A1) or 200 µg.L⁻¹ (A2); Glyphosate (G) with 65 µg.L⁻¹ (G1) or 6500 µg.L⁻¹ (G2); Atrazine and glyphosate (AG) group with 2 µg.L⁻¹ of atrazine associated with 65 µg.L⁻¹ of glyphosate (AG1), or 200 µg.L⁻¹ of atrazine with 6500 µg.L⁻¹ of glyphosate (AG2), and Control group (C) with distilled water. Three eggs were removed from each incubator on Day 30 and on Day 50 of incubation. Embryos were diaphanized with potassium hydroxide (KOH) and stained with Alizarin Red S and Alcian blue. Skeletal changes in embryos were not detected in the control group, but in 20 out of 36 (55.55%) exposed eggs: lack of sclerotic ossicles, changes in ossicle shape, fused or rudimentary ossicles and supernumerary ribs. This study concluded that atrazine at doses ranging from 2 to 200 µg.L⁻¹ and glyphosate at doses ranging from 65 to 6,500 µg.L⁻¹ induce skeletal malformations in *P. unifilis* embryos derived from exposed eggs.

SEMEN COLLECTION BY URETHRAL CATHETERIZATION AFTER IMMOBILIZATION WITH MEDETOMIDINE IN A FREE-RANGING GIANT ANTEATER (*MYRMECOPHAGA TRIDACTYLA*)

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The giant anteater is categorized as “Vulnerable” (IUCN, 2019) and implementation of assisted reproductive techniques, such as artificial insemination, might be critical for the conservation of this species. The present study describes the collection, evaluation and freezing of a semen sample in a wild giant anteater, captured in the Pantanal (Brazil). The animal was immobilized with an intramuscular injection of medetomidine (0,08 mg/kg body weight) and ketamine (5 mg/kg body weight), medetomidine inducing smooth muscle contraction in the vas deferens and thus resulting in the release of semen into the urethra. Subsequently, a tomcat urinary catheter was advanced into the urethra and semen was collected by capillary forces. An aliquot of the semen was diluted in OptiXcell® and frozen in liquid nitrogen. A semen sample of 0,2ml with a sperm concentration of $125 \times 10^6/\text{ml}$ was obtained. The fresh semen presented a sperm forward progression score of 4, sperm motility of 70% and 64% morphologically normal spermatozoa. Semen after thawing showed a forward progression score of 3, sperm motility of 30% and 30% morphologically normal spermatozoa, respectively. In comparison to semen obtained by electroejaculation in free-ranging giant anteaters (LUBA et al., 2015), the fresh semen sample in this study revealed lower volume, but similar sperm concentration and motility. Even though describing semen collection in only one specimen, the present study demonstrates that medetomidine administration and subsequent urethral catheterization is an effective method for semen collection in wild giant anteaters and could serve as an alternative to electroejaculation.

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ANATOMICAL ASSESMENT OF THE EXCRETORY SALT GLANDS OF THE LOGGERHEAD SEA TURTLE (*CARETTA CARETTA*) USING HIGH-FIELD MAGNETIC RESONANCE IMAGING

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The paired lachrymal salt glands allow sea turtles to maintain ionic homeostasis in a hypersaline environment by secreting a concentrated sodium chloride solution in response to increased plasma sodium. Several gland salt diseases have been reported in wild sea turtles. However, there is very little information about magnetic resonance imaging (MRI) features of these glands.

Our objective was to provide a thorough MRI description of the standard appearance of the excretory salt glands in three loggerhead sea turtles (*Caretta caretta*). MRI was acquired using a magnet operating at a field of 1.5-Tesla and a human coil. For determining data acquisition, with the animal placed in ventral recumbency, the MRI protocol was based on the use of Spin-echo (SE) T1-weighted and T2-weighted pulse sequences. Magnetic resonance images were taken in the transverse, sagittal and dorsal planes. Anatomical details were described according to the signal intensity of the MR images. The SE T1-weighted images showed an intermediate signal intensity in T1-weighted sequences compared to the low signal intensity in the T2-weighted images. The results of this MRI study provide a useful atlas with valuable anatomical information of the excretory salt glands in this sea turtle species, and may be useful for diagnosing disorders of these glands in sea turtles.

LOWER AUSTRIA - A HOT SPOT FOR ECHINOCOCCOSIS?

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Alveolar Echinococcosis is one of the most serious helminthic diseases of humans in the northern hemisphere. The WHO recently graded it as a neglected disease. The noxious agent is the fox tapeworm *Echinococcus multilocularis*. Humans serve as incidental hosts in the normally sylvatic parasitic cycle. Foxes (*Vulpes vulpes*), racoon dogs (*Nyctereutes procyonides*), racoons (*Procyon lotor*) and wolves (*Canis lupus*) are the usual final wildlife hosts for *E. multilocularis*. There are regional hotspots described in Switzerland, Germany, Austria and France. From Lower Austria and the city of Vienna 390 foxes were investigated from 2016-2018. Adult worms were diagnosed by using a modified sedimentation method ("shaking-in-a-vessel-technique").

The mean prevalence in all of the observed regions varied from 31 % (2016) to 22 % (2018) with no significant trend. Lower Austria is historically divided into four quarters, with every quarter being approximately the same size but all of them offering a different habitat for fox populations. Statistical analysis using ordinal logistic regression revealed a highly significant difference in the prevalence of *E. multilocularis* in red foxes regarding the individual quarters and the Viennese area. Therefore we conclude the existence of local hotspots for *E. multilocularis* inside the surveyed area.

MANAGEMENT OF A SEVERE HIP LESION IN A BREEDING MALE AMUR TIGER (*PANTHERA TIGRIS ALTAICA*)

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Amur Tigers are a critically endangered species, with only 500 individuals remaining in the wild, and 350 in European zoological institutions. Cliff, a male individual born in 2012, with high genetic value and was transferred to Besançon zoo in 2013 for breeding purposes. At 2 years of age, this tiger showed signs of hindlimb lameness. Several treatments were set up over a few month period, including a non-steroidal anti-inflammatory drug (Previcox®, Firocoxib 5 mg/kg, p.o.), opioids (Tramadol, 2 mg/kg, p.o.) and chondroprotective agents (Ekyflex®, harpagophytum, p.o.). Despite these medical treatments, lameness persisted, accompanied with amyotrophy. X-rays showed severe lesions of the right femoral head: acetabulum deformity, doubling twice in size, congruence default with femoral head showing an irregular surface associated with osteoarthritis. Considering the breeding value of this tiger and the involvement of hips during mating, a CT-scan was carried out to obtain a more precise diagnosis. Scanned images, interpreted by orthopaedic surgeons led us to consider a total hip replacement, but only with custom-made prosthetics. In order to ensure the feasibility of this surgery, prosthetics designers and manufacturing facilities specialized in surgical plates were approached, in addition to the consultation with the EEP's vet advisor and American experts in hip surgery on large felids. Due to the size and strength of Amur tigers, specialists reported side effects of total hip replacement (PAUL et al. 1985) such as luxation, fractures and death during post-operative care. For all these reasons, the surgery project on Cliff was aborted, especially since he coped quite well with the lameness. A chronic medical treatment plan was set up, adjusted by regular pain assessments and video monitoring. The success of this management took shape in 2017 with the birth of Cliff's first litter.

This case describes the management of a chronic lesion in an Amur tiger presenting a high genetic value within the critically endangered breeding program. After presenting diagnosis and therapeutic strategies, the authors raise the issue of animal welfare and staff security, both important topics which have to be considered when managing such a case.

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A CASE REPORT: A 1-WEEK-OLD MALAYAN TAPIR (*TAPIRUS INDICUS*) WITH HYDROCEPHALUS INTERNUS

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In July 2018 a 7-day old female Malayan tapir was reported less active but alert with tachypnea (30-50/min). In the next days, body temperature increased from 38.3°C to 39.6°C. Blood was collected and revealed anemia (PCV 0.13 l/l) and leucopenia ($1.1 \times 10^9/L$). Antibiotic and antiviral treatment was started (trimethoprim sulfadiazine 25 mg/kg BW SID IM, famcyclovir 8.8 mg/kg BW BID PO).

Two days later the tapir became recumbent, anorectic and showed muscle cramping and occasional protrusion of the membrana nictitans. Temperature varied between 37.5°C and 38.4°C. PCV was consistent but WBC increased ($3.7 \times 10^9/L$), and chemistry showed low total protein and albumin (52 g/L and 13 g/L) and low creatinine (58 $\mu\text{mol/L}$).

Based on the clinical signs the differential diagnosis was tetanus or meningoencephalitis. A wound was found on the hock of the animal, making tetanus more suspicious. Tetanus antitoxin (6 ml IV equine origin) was given immediately and antibiotics were switched to amoxycillin (21.5 mg/kg BW SC), since this is more effective against *Clostridia spp.* The animal was force-fed by an oesophageal tube.

Therapy was continued with antibiotics and tetanus serum the next day and paraffin per oesophageal tube and clysmata with sorbitol were started. The bladder was emptied by puncture through the abdominal wall, under ultrasound guidance.

A day later body temperature increased (39.6°C) and a continuous opisthotonos with high muscle tensions all over the body presented, therefore acepromazine 0.25 mg/kg BW IM - was given. The tapir unfortunately died within 12 hours after acepromazine was given. At necropsy, dilated ventricles in the brain fitted the diagnosis of hydrocephalus internus. No cause was found for the anaemia.

GENERALIZED CONVULSIVE SEIZURES AFTER CONTRAST-ENHANCED CT SCAN IN A RED KANGAROO (*MACROPUS RUFUS*) WITH DISCOSPONDYLITIS

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A five year old red kangaroo (*Macropus rufus*) was presented for hind limb ataxia and apathy. This animal had a history of chronic head tremors with unclear aetiology. Clinical examination and blood analyses were within reference ranges. The animal received trimethoprim/sulfamethoxazole injections (Septotryl®, Vetoquinol, Lure, France, 15mg/kg i.m. s.i.d. for 7 days) because toxoplasmosis was included a possible diagnosis. One week later, a lumbosacral cerebrospinal fluid (CSF) collection was performed under general anesthesia and analyses revealed normal cytology, negative bacterial cultures, and negative Toxoplasma PCR.

The animal was given dexamethasone (Dexafort®, Intervet, Beaucauze, France, 0,1mg/kg i.m. 2 injections one week apart), vitamin E/selenium (CertiselenE®, Theseo, Laval, France, 10mg/kg-0,02mg/kg p.o. s.i.d. for 10 days) and levamisole (Levamisole 3,75%®, Qalian, Segre, France, 7,5mg/kg p.o. s.i.d for 4 days) for treatment of possible nutritional myopathy or angiostrongylosis. Two weeks later, no improvement was observed and a contrast CT scan with a cerebellomedullary injection of 15 mL of iohexol was performed. As the contrast agent didn't reach the lumbar area, an additional lumbosacral injection of 5 mL of iohexol was performed.

CT scan images revealed a T4-T5 discospondylitis. During the recovery phase of anesthesia, the animal displayed generalized convulsive seizures. Despite diazepam injections (Valium Roche 10mg/2mL®, Roche, Boulogne Billancourt, France, 1mg/kg i.v. 2 injections one hour apart and one hour later 2mg/kg 2 injections two hours apart), the animal's condition deteriorated and the kangaroo was euthanized. Histopathologic examination revealed an atypical cerebral vascular proliferation.

Discospondylitis is well described in dogs and is an infection of two adjacent vertebral endplates and the associated intervertebral disk (KERWIN, 2014; DAVIS et al., 2000). Cases have also been described in a few wildlife species such as dolphins, armadillos, penguins, and wolves (ALEXANDER et al., 1989; NEVITT et al., 2018; BERGEN and GARTRELL, 2010; ZEIRA et al., 2013).

Complications, including seizures, can be associated with the administration of iodinated contrast agents such as iohexol are well described in dogs (DA COSTA et al., 2011). The risk of seizures is significantly higher after cerebellomedullary injections compared with lumbar injections and patient weight is significantly correlated with risk of seizures (BARONE et al., 2002).

Although discospondylitis has been described in several wildlife species, no data is available in macropods. Complications of myelography and the risk factors associated with it are also not well described in any wildlife species. The medical history and the histopathologic findings in the brain of this case raise questions about potential cerebral sensitivity as a predisposing factor for myelography-induced seizures.

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TRAINING AND VOLUNTARY ECHOCARDIOGRAPHY IN GIANT ALDABRA TORTOISES (*ALDABRACHELYS GIGANTEA*)

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We evaluated the feasibility of performing echocardiography in giant tortoises under trained, voluntary "restraint". No chemical sedation was used. Four giant Aldabra tortoises (*Aldabrachelys gigantea*), 2 adult males (250 kg and 220 kg) and 2 young females (75 kg and 85 kg), hosted in two zoological gardens in Italy, were trained to accept an echography probe inserted into the thoracic inlet for evaluation of the heart. We measured standard echocardiographic parameters and described echocardiographic characteristics by using both Doppler and B-mode. B-mode allowed the kinetic evaluation of the ventricles, the atria and the atrioventricular valves. Heart rate average was 21 ± 4 bpm (range 14-25 bpm). The averages of the diastolic and systolic areas index-linked to the subject weight were: $21 \pm 3 \text{ cm}^2$ and $9 \pm 1 \text{ cm}^2$. In the females, the aortic annulus diameter measured 11.2 ± 0.8 mm, while in the larger males, it measured 21.5 ± 0.3 mm. This experiment confirmed the feasibility of evaluating the cardiovascular system of giant tortoises using behavioural training. A larger number of patients will be necessary to further develop the echocardiography technique and establish "normal" standard references.

SHORT-TERM SURGICAL AND LASER THERAPY MANAGEMENT OF A SEVERE TRICHOMONOSIS IN A BONELLI'S EAGLE CHICK (*AQUILA FASCIATA*)

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Avian trichomonosis is an infectious disease caused by a flagellated protozoan parasite that affects the upper digestive tract of birds. Avian trichomonosis has been reported most frequently in young birds and has variable pathogenicity among avian species, ranging from asymptomatic infections to severe disease that ultimately causes the death of the animal.

A 45-days-old wild Bonelli's eagle (*Aquila fasciata*), a specimen included in the Life Bonelli's European conservation project, was removed from the nest and admitted into the wildlife rehabilitation center for further assessment. The eagle was presented with dehydration (10%), anorexia, weakness, hypothermia (38.9°) and severe dyspnea caused by large caseonecrotic lesions located in the oral cavity and the upper digestive tract.

The eagle was stabilized upon admission, and surgical intervention was performed in order to remove the granulomas that were causing difficulty in swallowing and breathing. Samples were collected for culture and PCR and the causative microorganism was identified as the protozoa *Trichomonas gallinae*.

To promote wound healing, laser therapy was employed as a coadjuvant to the medical treatment. Low Level Laser Therapy (LLLT) was applied using pulsed and continuous laser beam three times a week for three weeks. A dosage of 5J/cm² wound surface area was used.

Approximately one month after presentation, the fledgling eagle was successfully recovered and returned to the conservation program, where ultimately it was released back into the wild.

This is the authors' first-time applying LLLT as an *adjuvant therapy* to promote post-operative wound healing after removal of granulomas caused by the protozoa *Trichomonas gallinae*. According to the authors' experience, the combination of *laser* therapy and post-operative wound care have a faster and greater effect on wound healing, if compared with common therapies used previously on cases with *Trichomonas gallinae* infection.



Fig. 1: Multiple granulomas in oral cavity. Severe glottis collapse.



Fig. 2: Removing sublingual granuloma.



Fig. 3: Removing palatine granuloma.



Fig. 4: Oral cavity after surgical removal of granulomas.



Fig. 5: Day when the animal was released.

SEPTIC ARTHRITIS OF THE SHOULDER IN A BABY GIRAFFE (*GIRAFFA CAMELOPARDALIS*): A CASE REPORT

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A 3-month-old female giraffe (*Giraffa camelopardalis*) born in captivity at a zoo in Fuerteventura, Spain, was rejected by its mother and hand-raised by the keepers. The giraffe was fed with fresh cow milk and received frozen camel colostrum the first 24h.

The animal was noted to have mild right forelimb lameness. It was observed for a few days without treatment due to suspicion of trauma. One week later the lameness did not disappear by itself, so flunixin meglumine (1.1 mg/kg BW) was administered subcutaneously SID for 5 days. At the same time radiography was performed without abnormalities.

The giraffe showed improvement during treatment, but the lameness reappeared when treatment was discontinued. After a few weeks, the shoulder joint started to be hyperthermic, and was swollen and painful.

Ultrasound-guided needle aspiration was performed, and purulent liquid was evacuated from the joint. The microorganism isolated by culture was *E. coli*. Catheter lavages with saline solution and amikacin (1 g) as local antimicrobial therapy by direct intra-articular injection were performed twice a week, as well as cefquinome (1.0 mg/kg BW) as systemic antibiotic was administered intramuscularly SID, both based on culture. Initially, the giraffe showed a remarkable improvement but after a short term, treatments started to be less effective.

Roughly 2 months after initiation of therapy, the patient showed similar symptoms in both coxofemoral joints. Therefore, the giraffe was euthanized. Histopathology revealed severe chronic fibrinous arthritis most probably induced by bacterial infection.

Approximately 3 weeks before the first limping episode, the animal had presented with diarrhea and had been treated with the systemic antibiotic penicillin (20,000 IU/kg BW) administered intramuscularly SID twice at 72h interval, based on culture. The isolated bacteria were identified as *E. coli*.

The authors suggest that, as in many different species, where the most common source of bacteria is hematogenous transfer to joints from the lungs, intestines or umbilical structures, this may have been also the case in this particular patient. Furthermore, the quality and absorption of colostrum were not optimal making it more susceptible to diseases.

SUSPECTED NEPHRITIC COLIC IN AN ORANG UTAN (*PONGO PYGMAEUS* *SSP. PYGMAEUS*)

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An 11-yr-old captive born female orang utan (*Pongo pygmaeus ssp. pygmaeus*) presented with sudden apathy and anorexia. Acute cystitis (leucocyturia) was diagnosed from urine analysis sampled from the enclosure. After a first medical treatment (dexamethasone 2 mg (0.042 mg/kg BW), danofloxacin 360 mg (7.7 mg/kg BW), IM, once) her condition immediately improved. One month later, she presented again with apathy. Leucocytes were present in the urine sample. The animal received fosfomycine (3 mg (0.064 mg/kg BW), PO, once) followed by phloroglucinol (126 mg (2.7 mg/kg BW), PO, BID, for 3 days) and meloxicam (30 mg (0.63 mg/kg BW), PO, SID, for 3 days). The week after, she was anesthetized (ketamine 3.8 mg/kg and medetomidine 13 µg/kg BW) for further investigations. Urinalysis (by catheterization) showed rare leucocytes, no stones. Bacteriology was negative. Ultrasound examination revealed a hypertrophy of the bladder mucosa, a right nephromegaly with a lobulated anechoic image in continuity with the kidney. The differential diagnoses at this time were hydronephrosis, pyelonephritis, kidney neoplasia. Despite the improvement of her condition, a CT examination was scheduled. The main lesions were a right renal hypoperfusion with irregular shape and a marked ureteric dilation. The lesional computertomographic features were compatible with a possible right renal post-obstructive syndrome without however, thus far, evidence of any identifiable ureteric lithiasis. There were no formal computertomographic findings in favour of a neoplastic hypothesis.

This case is interesting for the unexpected lesion observed in the right kidney and, for the authors, is the first description of a renal post-obstructive syndrome suspected to be secondary to a nephritic colic in an orangutan. Today, six months later, the animal is in good health and shows normal behaviour.

INTEGRATED MANAGEMENT OF A CAPTIVE L'HOEST'S MONKEY (*ALLOCHROCEBUS LHOESTI*) WITH HUMERAL FRACTURE

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For major orthopaedic procedures in zoo animal species, cooperation between zoo veterinarians, animal caretakers, anaesthetists, and surgeons is crucial to achieve successful therapeutic results. An adult male L'Hoest's monkey (*Allochrocebus lhoesti*), weighing 9.3 kg from the Parco Natura Viva, Bussolengo-Italy presented a comminuted, metaphyseal fracture of the left humerus. The zoo staff immediately provided antibiotic prophylaxis (ceftazidime, 25 mg/kg BW IM SID) and pain therapy (tramadol 2 mg/kg BW PO SID). The patient was referred to the University of Padova Veterinary Teaching Hospital where a team of anaesthesiologists and surgeons performed the internal fixation of the humeral fracture under isoflurane anaesthesia. Remifentanyl (0.25 mcg/kg BW/min) and ketamine (10 mcg/kg BW/min) infusion provided intraoperative analgesia. The smooth, rapid and full recovery from anaesthesia allowed to bring the animal back to the zoo one hour after the end of the surgery. In the postoperative period the monkey was restricted in a cage but in contact with the family through a grate. Antibiotic (ceftazidime, 25 mg/kg BW IM BID for 4 days, then cefixima, 25 mg/kg BW PO SID for 15 more days) and pain therapy (tramadol, 2 mg/kg BW PO BID for 7 days and meloxicam, 0.1 mg/kg BW PO SID for 15 days) was also provided. A form to record food intake, posture, alertness level, physiological functions, limb movements and pain signs was filled daily by keepers under the supervision of the zoo veterinarian. Five days post-surgery the animal started to move the arm and at day 23 the monkey showed complete functional recovery. Radiographic examination revealed bone consolidation and the animal was reintroduced within its group. In conclusion, this case highlights how timely intervention, adequate pain management, constant postoperative monitoring and cooperation are essential for the positive outcome of major trauma in non-human primate.

THREE CASES OF FATAL CARDIAC LISTERIOSIS IN INLAND BEARDED DRAGONS (*POGONA VITTICEPS*)

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Listeriosis is of particular importance for pregnant women and domestic ruminants. Of the recognised species, *Listeria monocytogenes* and *L. ivanovii* are important pathogens. These Gram-positive rods incite septicaemia, encephalitis and abortion. Overt clinical disease is rare in reptiles, but in one survey, faecal carriage of *Listeria* was found in 1.3% to 30% of clinically healthy tortoise and snake species (WEBER, 1993).

Our study summarises pathological findings in three inland bearded dragons diagnosed with fatal listeriosis in 2010 and 2018, respectively. All animals originated from the same collection. Signalement, clinical signs, treatment and culture results are outlined in Table 1. Information on the diet was not available for any case.

Table 1	Age	Sex	Clinical signs	Treatment	Microbiology
Case 1	18 months	Male	Not reported	None	<i>Listeria ivanovii</i>
Case 2	16 months	Male	Not reported	Marbocyl	Not available
Case 3	4 years	Female	Sudden death	None	<i>Listeria monocytogenes</i>

Infection resulted in severe chronic-active granulomatous peri-, epi- and myocarditis with intralesional Gram-positive bacteria, granulomatous arteritis (case 2) and pericardial effusion, marked oedema, and septic spread. Findings are similar to two previously reported cases in a 1 year old female and a 2.5 year old male inland bearded dragon (GIRLING and FRASER, 2004). These animals showed clinical lethargy, dyspnoea and oedema (periorbital and throat region). One animal had been fed thawed newborn mice.

This case series demonstrates that listeriosis should be considered in bearded dragons presenting with oedema and dyspnoea. The propensity for cardiac disease is unusual but appears pathognomonic in this species. Though faecal carriage has been demonstrated, susceptibility to food-borne infection appears likely and constitutes an important dietary consideration. Alongside Salmonellosis, *Listeria* carriage and disease presents a zoonosis risk in pet bearded dragons.

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USE OF HERBAL MEDICINE AS COMPLEMENTARY TREATMENT IN ZOO SPECIES

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The use of herbal medicinal products and supplements has increased over the past three decades. (EKOR, 2014) In numerous cases, the use of complementary and alternative medicines (CAMs) is interesting in a modern multimodal approach, including for zoo animals, but much of this documentation is anecdotal. (HOBY *et al.*, 2015) However, these substances are often viewed as a balanced and moderated approach to healing and causing less adverse effects than allopathic drugs. (EKOR, 2014) More than just an alternative pharmacopoeia, they represent an individualized, holistic approach.

Case 1: A 26-year-old male blue and yellow macaw (*Ara ararauna*) presented severe apathy. Under general anesthesia with isoflurane by mask, an extensive clinical assessment, including blood chemistries, radiographs, and endoscopy, was performed. The bird presented an abnormal cardiac silhouette and dilation of great vessels. Advanced imaging by computed tomography confirmed severe arteriosclerosis affecting the brachiocephalic artery and identified clinically relevant bilateral arthrosis of the tibiotarso-tarsometatarsal. Initially, the animal received imidapril (0.2 mg/kg PO SID) to address presumptive systemic arterial hypertension and meloxicam (0.5 mg/kg, PO, SID, 5 days) for analgesia. To replace the NSAID treatment long-term, a mix of standardized plant extracts was prescribed that was composed of hawthorn (*Crataegus* sp.), blackcurrant (*Ribes nigrum*), olive (*Olea europaea*) and valerian (*Valeriana officinalis*) in equal proportions (1mL/kg, PO, SID for 3 weeks). Hawthorn contains positive inotropic and dromotropic agents, and negative chronotropic and bathmotropic agents that were considered relevant in this case to reduce systemic hypertension. Additionally, a reduction in blood lipids was expected. (GUO *et al.*, 2008; MAY, 2014; PITTLER *et al.*, 2003; RIGELSKY AND SWEET, 2002) Blackcurrant has potent anti-inflammatory action. (BENN *et al.*, 2014; BONARSKA-KUJAWA *et al.*, 2014; DECLUME, 1989; LEE AND LEE, 2019; MAY, 2014; SHAW *et al.*, 2017) Olive leaf extract is anti-inflammatory and anti-hypertensive agent and reduces systemic cholesterol. (EL AND KARAKAYA, 2009; KHAYYAL *et al.*, 2002; MAY, 2014) Valerian is analgesic, anxiolytic, hypnotic and sedative which was relevant to address the stress displayed in this bird's initial presentation and attributed to a recent hierarchical reorganization of the group. (ANDREATINI *et al.*, 2002; HILLER AND ZETLER, 1996; MAY, 2014; YUAN *et al.*, 2004; FERNÁNDEZ *et al.*, 2004) This treatment combination improved the quality of life for the animal, and within a week, the bird became less apathetic.

However, after the first 3 weeks of treatment, the bird was non-compliant with the full dose (1mL) on a daily basis. Despite the efficacy of the treatment, it was decided to change to gemmotherapy, or a form of herbal medicine that uses remedies made principally from the embryonic tissue (buds and emerging shoot) of various trees and shrubs. (VIRIOT, 2015; LEDOUX *et al.*, 2012; MAY, 2014; TÉTAU, 1987) This concentrated approach reduced the daily dose to one drop (<0.1mL) and required administration only three weeks a month. During the fourth week of the month, the treatment is stopped to avoid habituation phenomenon and permit a clinical evaluation. The selected combination included hazel (*Corylus avellana*), common juniper (*Juniperus communis*), blackcurrant (*Ribes nigrum*) and mountain pine (*Pinus montana*). Hazel has vaso- and arthro-protective effects, sedative action, and reduces systemic cholesterol. Common juniper is cholesterol-lowering and regulates glycemia, and is hepato- and reno-protective. Mountain pine has joint tropism that stimulates chondrocytes and improves the bone protein matrix. (ANDRIANNE, 2008; VIRIOT, 2015; LEDOUX *et al.*, 2012; MAY, 2014;

TÉTAU, 1987) After only one week of this modified treatment, the macaw again no longer showed any clinical signs of apathy. It has remained on this treatment for 7 months without signs of illness.

Case 2: A 17-year-old male Sichuan takin (*Budorcas taxicolor tibetana*) male presented chronic diarrhea for several years. Regular coproscopic evaluations diagnosed successive severe infestations with *Giardia* sp., *Eimeria* sp., or strongyles. Despite several treatments including netomibin (10 mg/kg PO once), fenbendazole (10 mg/kg PO SID 3 days) and toltrazuril (20 mg/kg PO once), the diarrhea was not resolved. A phytotherapeutic protocol was developed to manage clinical signs and improve animal welfare. A mixture of liquorice (*Glycyrrhiza glabra*), Eastern purple coneflower (*Echinacea purpurea*), great yellow gentian (*Gentiana lutea*) and common lady's mantle (*Alchemilla vulgaris*) was administered orally at 15 mL BID the first week of each month. The chemicals contained in liquorice are anti-inflammatory, decrease mucus secretions and have bacterial and viral anti-adhesion properties. Purple coneflower is immunomodulant, specifically increasing IL-1, IL-3, IL-6, and antibacterial. Gentian is gastroprotective, antibacterial, eupeptic and antispasmodic. (ROJAS et al., 2000). Common lady's mantle is astringent, angioprotective and has promitotic activity in epithelial cells and myofibroblasts. Within a few days, this treatment stopped diarrhea, except for rare relapses, although it is maintained monthly.

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IMPACT OF SITE ON MESENCHYMAL STEM CELL YIELD IN CHICKENS (*GALLUS GALLUS DOMESTICUS*)

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Bone marrow mesenchymal stem cells (BM-MSCs) are multipotent stromal cells isolated from bone marrow that can differentiate into a variety of cell types including osteoblasts, chondrocytes, and adipocytes. MSCs may have a role in the repair and regeneration of damaged bone, tendon, and cartilage. Previous studies demonstrate improvement of tendonitis, osteoarthritis, and cartilage injury in veterinary patients utilizing treatment with MSCs. The previously reported methods of harvesting MSC's from chickens involved euthanasia and bone transection to obtain bone marrow samples. A non-lethal method of obtaining BM-MSC's is crucial if this technology is to be used in endangered or threatened avian species. The objective of this study was to evaluate MSC yield from chicken bone marrow via bone marrow aspirates of the keel and proximal tibiotarsus. These yields were compared to MSC yield using the previously described, lethal technique of flushing bone marrow from the contralateral tibiotarsus. Colony forming unit (CFU) yield obtained by tibiotarsal aspiration was significantly greater for passage 1 than from keel aspirate or tibiotarsal flushing ($p=0.029$). Seeding density also had an impact on CFU numbers with higher CFU yield found with higher plate seeding density at passage 1 ($p=0.0003$) and 3 ($p=0.0004$). Results indicated that MSCs can be successfully isolated from chicken bone marrow using tibiotarsal aspirates, sparing euthanasia of the animal to obtain samples. This non-lethal method of obtaining MSCs has potential benefit to all avian species, including those that may be threatened or endangered.

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RETROSPECTIVE EVALUATION OF *ERYSIPELOTHRIX RHUSIOPATHIAE* INFECTIONS AND ITS ZOOBOTIC POTENTIAL IN MARINE MAMMALS OF GERMAN WATERS

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Erysipelothrix rhusiopathiae is an ubiquitous bacterium with zoonotic disease potential, which even survives in the marine environment. Between 1990 and 2015, necropsies including microbiological analyses (cultures) were performed on 865 harbour seals (*Phoca vitulina*), 86 grey seals (*Halichoerus grypus*) and 536 harbour porpoises (*Phocoena phocoena*) within a stranding monitoring in Germany. The animals either stranded dead along the Baltic and North Sea coastlines of Schleswig – Holstein or were accidentally caught by fishermen. Retrospectively *Erysipelothrix rhusiopathiae* could be cultured in 7,2% [n=62] of harbour seals, 9,3% [n=8] of grey seals and 6% [n=32] of harbour porpoises. All age classes were affected within all three species. The bacterium was isolated from one to eight organs per animal with various quantity. No preferences regarding the sex and origin of the animals were seen. Every carcass was handled by humans but just a few showed typical macroscopic skin lesions. In 2013, a seal ranger developed severe septicaemia after retrieving a carcass of an adult male grey seal from the Baltic Sea. *Erysipelothrix rhusiopathiae* was obtained from the lesions and considered the cause of disease. While direct zoonotic transfer could not be proven, the pathogen was cultured from the skin of the dead grey seal amongst other bacteria. The study results contribute to the advancement in preventive protection of public health. While the overall zoonotic transmission risk may be low, the above mentioned case example underlines the necessity and consistent implementation of suitable safety precautions in all kinds of dealing with marine mammals.

REACHING BEYOND THE OBJECTIVES OF A DORCAS GAZELLE REINTRODUCTION PROJECT

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A 5 year project to reintroduce this dorcas gazelles (*Gazella dorcas*) to Senegal began in 2007. Its goals were to establish an ongoing reintroduction program of this species to a previously fenced area, provide technical training for staff involved in the project, and enhance awareness for the species in particular and environmental conservation among the local population. To these ends, an adaptation and breeding facility was established in NE Senegal (RSF Guembeul) with 26 *G. dorcas* transferred from various EEP holders. From there, 34 gazelles have been introduced into a 1200 ha fenced area within the Réserve de Faune du Ferlo Nord. An abortion epidemic at RSF Guembeul interrupted gazelle transfers from the breeding center from 2011 to 2017. While this was a major setback, it led to extended work and on-site visits by the project team, which resulted in a substantial increase in the impact of the project by 2017. This included setting up of a local wildlife diseases network, coaching more than 40 Senegalese personnel, as well extensive engagement with the local population in Ferlo, with awareness-building extending to their natural heritage and sustainable use of the reserve's natural resources, increased education opportunities, and fostering working relations with the environment authorities. Finally, as a result of Barcelona Zoo's sustained engagement in this project, the Sahel has become a cornerstone of the zoo's master plan; a Sahelian savannah is currently under development, and Sahelian endangered species will be central to the zoo's ex-situ and in-situ conservation projects.

REPRODUCTIVE MORPHOLOGY OF ADULT MALE AND FEMALE GIANT ANTEATERS (*MYRMECOPHAGA TRIDACTYLA*)

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The giant anteater is categorized as “Vulnerable” (IUCN, 2019) and understanding of its reproductive characteristics is critical for the species’ conservation. The present study evaluates gross and microscopic anatomy of the reproductive organs of 33 male and 25 female adult road-kill specimens in Brazil. Adult male giant anteaters present a short conical penis, intraabdominal testes and prostate, vesicular and bulbourethral glands. Furthermore, a well-developed remnant of the paramesonephric duct forms a tubular structure, which extends from the seminal colliculus through the genital fold and attaches bilaterally on the body of the epididymis. Histologically, this structure presents features of the female vagina, uterus and uterine tubes and seems to have secretory functions. In adult female giant anteaters, a simple uterus is observed, which is directly continuous with the vaginal canal, without forming a cervix. In addition, females present mesonephric duct remnants: two thin ducts pass through the ventral vaginal and uterine wall running alongside the uterine tubes to the hilus of the ovaries. Histologically, a rete ovarii and seminiferous cord-like structures were observed in the ovaries. In the urethra, close to the neck of the bladder, glandular structures were found and a pair of major vestibular glands, comparable with bulbourethral glands in males, opens into the vaginal vestibule. Thus, the reproductive organs of both male and female giant anteaters reflect some characteristics of the opposite sex. The supposedly secretory function of these vestigial structures still needs to be clarified and might have importance for the implementation of assisted reproductive techniques.

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ACUTE MYCOPLASMOSIS IN PANTHER CHAMELEON (*FURCIFER PARDALIS*, CUVIER, 1829) STOCK

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The authors diagnosed *Mycoplasma* infection in a panther chameleon (*Furcifer pardalis*) stock. The owner has introduced newly imported reptiles from a Madagascanian reptile farm to his own stock without quarantine. The animals suffered from cold temperature after a long power failure. Loss of appetite and intensive mucous content in the oral cavity were observed. The chameleons died 3-4 days after the clinical signs had appeared. During the necropsy a small amount of foamy content was found in the lung. Large amount of mucous and turbid contents were observed in the oral cavity and the trachea. Gross pathological changes were not detected at necropsy. Oral cavity swabs were collected from chameleons in 2016. Swabs were washed in 2 ml Friis broth medium and incubated at 25°C until colour change (24 hour). When colour change of the broth media occurred cultures were inoculated into solid media and incubated until visible colonies appeared. *Mycoplasma* strains were once filter-cloned, and DNA extraction was performed from the pure cultures using QIAamp DNA mini kit (Qiagen Inc., Hilden, Germany) according to the manufacturer's instructions. A universal *Mycoplasma* PCR system targeting the 16S/23S rRNA intergenic spacer region in *Mycoplasmatales* followed by sequencing on an ABI Prism 3100 automated DNA sequencer. BLAST search and sequence analysis were performed in order to identify the isolated *Mycoplasma* species. Two *Mycoplasma* strains were isolated from the samples. They most related species based on the BLAST search are *M. crocodyli* (95% identity) and *M. iguanae* (87% identity).

AMYLOIDOSIS IN NINE CARCALS (*CARACAL CARACAL*) FROM EUROPEAN ZOOLOGICAL INSTITUTIONS

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Amyloidosis is a disease in which amyloid, a homogenous proteinaceous material, is deposited extracellularly. Due to pressure atrophy of the affected cells and depending on the organ involved, amyloidosis may present with various clinical forms and lesions. Amyloidosis is an important disease among captive cheetahs (*Acinonyx jubatus*) and has been reported in Siberian tigers (*Panthera tigris altaica*) and in black-footed cats (*Felis nigripes*) (SCHULZE et al. 1998, TERIO et al. 2018). Nine caracals (*Caracal caracal*) from three different institutions were evaluated histopathologically. The 6 males and 3 females died between 2008 and 2018 at a median age of six years \pm 2.5 months (median \pm IQR). Seven out of nine animals suffered from acute renal failure with weight loss, lethargy, anorexia, dehydration, and azotemia. The main gross lesion was pallor of the renal cortex. In two out of nine animals, amyloidosis was an incidental post-mortem finding, one animal showed severe pancreatic amyloidosis mainly affecting the exocrine pancreas. Histologically, glomerular amyloidosis was present in all nine animals, and was the predominant renal manifestation of amyloidosis. Additional findings included splenic amyloidosis (8/8), amyloid in the lamina propria of the intestine (5/5) and in the lingual submucosa (4/4). Gastric mineralization was present in four animals suffering from renal failure. The aetiology of the amyloidosis remains unknown, however several of the animals were related, which might suggest a familial trait. Amyloidosis should be considered as a significant disease in the caracal. Especially in renal failure it should be included as a major differential diagnosis.

Acknowledgements

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CONCENTRATION ON CANCER – ESCRA DATABASE UPDATES AND HOW YOU CAN HELP

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The Exotic Species Cancer Research Alliance (ESCRA) Database (www.escra.org) launched over two years ago in the United States of America. Since that time we have accumulated the participation of over 75 US zoos and aquariums, private practices and universities. We have over 700 records of cancer thus far. This database is focused not only on finding out which cancers are common, it is also examines age, sex, reproductive history, treatments, and survival post-diagnosis. The most prevalent cancers in our database at this time are hepatocellular carcinoma, lymphoma, and squamous cell carcinoma. The most commonly represented species include the domestic ferret, guinea pig, the fat tailed dwarf lemur, Coquerel's giant mouse lemur, mongoose lemur, gray mouse lemur, and budgerigars. We have also found that cancer severity appears to be increased in snakes, and that some species of big cats such as tigers and lions seem prone to developing lymphoma. We are continuously recruiting retrospective and prospective cancer cases, and are available to assist in treatment recommendations for prospective cases. With continued case accrual, we will not only have improved characterization of the most common cases, but we will have improved evidence to recommend therapeutics and provide more accurate survival predictions beyond the typically referred to human or domestic animal survival times. Through our work, we are able to calculate improved survival times that apply more specifically to our zoological animals.

EVALUATION OF THE MASIMO SEDLINE® ELECTROENCEPHALOGRAM FOR ANAESTHESIA MONITORING IN AN OLD COMPARED TO A YOUNG TIGER (*PANTHERA TIGRIS*)

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In zoological medicine monitoring the depth of anaesthesia is crucial, not only for animal welfare aspects, but also for personnel safety reasons. Brain function monitors have improved safety and efficiency in human anaesthesia. This study evaluated the use of the electroencephalogram (EEG)^a for anaesthesia monitoring in a 15- and a 4-year-old tiger. Both females were clinically healthy and were immobilised for post-treatment check-ups using ketamine^b, medetomidine^c, midazolam^d and butorphanol^e. During anaesthesia patient state index (PSI), suppression rate (SR), and spectral edge frequency (SEF) were collected. Data were statistically analysed using linear mixed effect models. The older tiger showed a significant higher SR than the younger tiger, which indicates the proportion of time in electrical silence was significantly greater (regression coefficient = 89.27, $p < 0.001$). Recovery in the geriatric animal was extended. This may be due to altered sensitivity to anaesthetics in elderly patients and has been described in humans. In both tigers PSI was negatively correlated to SR (old: $r = 0.77$, $p < 0.001$; young: $r = 0.53$, $p < 0.001$; linear regression). The range of the SEF throughout the defined anaesthesia period was similar in both tigers and appeared to be a reliable parameter for the depth of anaesthesia.

There is a high potential for the EEG to be used for anaesthesia monitoring in zoological medicine, but further studies in this field are needed. Like in human medicine, elderly patients seem to require less anaesthetics to achieve the same level of anaesthesia as their younger conspecifics and to experience better recoveries. Age-dependent anaesthesia balancing should be evaluated more carefully.

^aMasimo SedLine®, Masimo Coporation, Irvine, CA, USA

^bKetamidol® 100 mg/ml, Richter Pharma AG, Wels Austria, 3 mg/kg IM

^cMedetomidine 20 mg/ml, magistral formulation, Vienna, Austria, 0.035 mg/kg IM

^dMidazolam 50 mg/ml, magistral formulation, Vienna, Austria, old: 0.05 mg/kg IM, young: 0.1 mg/kg IM

^eAlvegesic® vet. 10 mg/ml, Alvetra u. Werfft GmbH, Vienna, Austria, 0.05 mg/kg IM

GLENOID DYSPLASIA AND BICIPITAL TENOSYNOVITIS IN A TIGER (*PANTHERA TIGRIS*)

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This case report describes a rare case of glenoid dysplasia and bicipital tenosynovitis in a tiger (*Panthera tigris*). The neutered 5-year-old female (131 kg BW) presented with chronic intermittent weight bearing lameness of the right forelimb, which first appeared at about 12 months of age. An oral treatment with Firocoxib 5 mg/kg SID (Previcox® 227 mg, Merial, France) over 7-10 days, in combination with Omeprazole 40 mg SID (Omeprazol-ratiopharm® NT 40 mg, Ratiopharm, Ulm, Germany), was given whenever lameness was presented, but did not noticeably improve the situation. Mild degenerative changes of the glenohumeral joint with shell formation at the supraglenoid and infraglenoid tubercle were seen radiographically, as well as mild calcifications at the insertion of the biceps tendon. A CT scan revealed a high degree of osteophytosis at all regions of the joint. The medial joint space was irregularly enlarged. The subchondral bone of the glenoid cavity was highly abrasive and showed significant subchondral defects. In the intertubercular groove of the humerus mild to moderate shell-like periosteal reactions were visible. The findings were consistent with a severe degenerative joint disease of the right shoulder. The cause could not be determined. Because of the flattening of the medial part of the glenoid cavity a dysplasia was suspected. The tiger was treated with a single intra-articular injection of 24 mg betamethasone acetate (Celestovet® 12 mg/ml, MSD Animal Health, Germany), which resulted in the animal being free from lameness for four weeks. Then, intermittent lameness recurred. As this is a rare condition in cats, there are no reports on conservative or surgical treatment outcomes neither in cats nor in tigers. A repeated treatment with betamethasone or arthroscopy of the joint and removal of the osteophytes might be an option. Long-term joint supportive treatment and pain management will be necessary in any case.

NON-SPECIFIC REACTIVE HEPATITIS ASSOCIATED TO POLLUTANTS IN BOTTLENOSE DOLPHINS (*TURSIOPS TRUNCATUS*) STRANDED IN THE CANARY ISLANDS

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This retrospective study describes non-specific reactive hepatitis (NSRH) in 49 tursiops from a total of 145 cetaceans stranded in the Canary Islands between 2002 and 2010. NSRH was marked by the proliferation of Kupffer cells and granulocytes, plus mononuclear cells scattered throughout the liver parenchyma and in the portal stroma. Blubber and liver samples were analysed for organochlorine polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDTs). All animals showed different concentrations of pollutants in the blubber and liver. The toxicological study revealed that PCBs were found in the greatest concentrations. PCB levels in the liver were lower than those found in the blubber. Similar PCB congener compositions were observed in the two tissues, with a relatively high contribution of hexa- and hepta-chlorobiphenyls. Within the organochlorine pesticide compounds, the DDT groups were present in the highest concentrations. p,p'-DDE was the DDT-group compound present at the highest concentration in the two tissues analysed. Two animals with severe NSRH showed the highest PCB congener and organochlorine pesticides concentration, which could be related to the pathogenesis of this process. The PCB and DDT levels found in the blubber and livers of our animals were considered to be in the medium range in comparison with previous studies from other dolphin populations. It is reasonable to assume that these concentrations could have adverse effects on their health status, but their hepatotoxicity cannot be established. This is the first study that analyzes the severity of NSRH and the levels of pollutants in the blubber and liver of cetacean stranded in the Canary Islands. Studies in larger series of animals must be conducted to learn more about the physiological effects of these pollutants in marine mammals.

GROSS AND MAGNETIC RESONANCE IMAGING STUDY OF THE FEMOROTIBIAL JOINT IN A NORMAL BENGAL TIGER (*PANTHERA TIGRIS*)

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This study describes the normal appearance of the structures of the stifle joint of a Bengal tiger (*Panthera tigris*) using magnetic resonance imaging (MRI) and gross anatomical dissections. A cadaver of a mature female was imaged using specific sequences as the Spin-echo (SE) T1-weighting and Gradient-echo (GE) STIR T2-weighting sequences in sagittal, dorsal and transverse planes, with a magnet of 0.5 Tesla. The bony and articular structures were identified and labelled on anatomical dissections, as well as on the magnetic resonance (MR) images. SE T1-weighted sequence provided excellent resolution of the subchondral bones of the femur, tibia and patella compared with the GE STIR T2-weighted MR images. Articular cartilage and synovial fluid were visualised with high signal intensity in GE STIR T2-weighted sequence, compared with SE T1-weighted sequence, where they appeared with intermediate intensity signal. Menisci and ligaments of the stifle joint were visible with low signal intensity in both sequences. The infrapatellar fat pad was hyperintense on SE T1-weighted images and showed low signal intensity on GE STIR T2-weighted images.

This study provided adequate information of the Bengal tiger stifle joints that can be used as initial anatomic reference for interpretation of MR stifle images and to assist in the diagnosis of diseases of this region.

HEPATIC FAILURE IN DOLPHINS AS CONSEQUENCE OF *CAMPULA* INFESTATION

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Hepatic failure produced by *Campula* spp. is described in one striped dolphin (*Stenella coeruleoalba*) and 4 harbour porpoises (*Phocoena phocoena*). The hepatic findings observed on gross necropsy and histology confirmed the diagnosis of biliary cirrhosis produced by *Campula* spp. The lesions showed severe proliferation of fibrous connective tissue with loss of the lobular pattern, nodular regeneration of the hepatic tissue, bile duct hyperplasia and severe inflammatory infiltrate composed of eosinophils, lymphocytes and plasma cells. These lesions were associated with severe infestation by the parasite and the presence of large number of their eggs in the stroma. This trematode has frequently been found in the liver and pancreas of cetaceans. Typically, infestation is light and may be asymptomatic. In humans and ruminants, heavy infestation may cause changes similar to those observed here where parasite eggs in the stroma of portal spaces, causes severe granulomatous lesions and destruction of the biliary ducts. This is the first report of biliary cirrhosis associated with *Campula* spp. in cetaceans. While these type of lesions have been well documented in ruminants with fascioliasis and in humans with biliary trematodiasis, similar level of severity have not typically been found in cetaceans with this parasite. It seems that the massive infestation by this parasite should be considered a differential diagnosis of hepatic failure resulting in stranding of marine mammals.

HAND-REARING IN ASIAN ELEPHANTS (*ELEPHAS MAXIMUS*) – A CASE REPORT

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Neonatal mortality is one of the main causes of calf losses in the Asian elephant (*Elephas maximus*) captive population, for example, due to the aggressive behaviour of the mother towards the newborn leading to reduced or no nursing, leaving the zoos with the long and arduous task to bottle-feed the calves or, to do not hand-rear and “prepare for calf loss”.

On 2011, Vishesh (0.1), calved a most likely premature male calf, born underweight. The cow rejected the calf, presented offensive behaviour towards her offspring, and forbade breastfeeding. Despite all the efforts made by the caretakers, the calf was not gaining weight and died under 2 months of age. On the 4th of February 2014, the dam gave birth to her second calf – Sumitra (0.1). Sumitra was not able to nurse either, also due to lack of milk production by the mother and therefore, the decision was to hand-rear and attempt to save the calf.

Although milk replacement formulas are positively used in range countries and have helped to rescue several orphaned calves to the point of release and reintroduction, so far, in Europe, there is no report case of an Asian elephant calf, bottle fed from the first day of life, that had survived to reach adulthood. Therefore, there is no good milk substitute formula in the market for captive Asian elephant calves to succeed in case of rejection or the death of the mother.

Here we present a case report of a hand-reared female calf, that presented good growth rates, with a stable body weight increase, for nearly 2 years of age, until the moment she succumbed to the devastating elephant endotheliotropic herpesvirus (EEHV). This Herpesvirus is considered the major cause of calf mortality in the captive Asian elephant population. EEHV haemorrhagic disease is responsible for the death of nearly one-third of all deaths in Europe, which is further aggravated in as much as 75% of the fatal cases occurs between 1.4 and 3.2 years of age, wherefore, a serious risk to the conservation breeding efforts made by zoos.

On necropsy, besides EEHV common lesions, Sumitra presented a very good body condition, and her bones were considered unusually well-developed when comparing to previous cases, not successful in hand-rearing. Computer Tomography findings also revealed good bone density and calcification state.

Therefore, the milk formula developed had worked well and covered the nutritional growing necessities of the rejected calf. A next step will be to identify which is the missing component that allows us to protect the calves from EEHV.

Although Sumitra was not able to be saved, we acquired new knowledge from her and will hopefully help other elephant calves in a hand-rearing situation to thrive and achieve adulthood.

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GASTROINTESTINAL PARASITES IN PRZEWALSKI HORSES (*EQUUS FERUS PRZEWALSKII*) AT HORTOBÁGY NATIONAL PARK, HUNGARY – PRELIMINARY RESULTS

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Summary

A collection of 79 faecal samples was performed between September and November 2018 from a population with approximately 280 Przewalski horses (*Equus ferus przewalskii*) from the Pentezug Wild Horse Reserve. The coprological methods (McMaster, Willis flotation, natural sedimentation, Baermann and coproculture) and microscopical identification were performed in all the samples. Results show an average level of 1287 Eggs per Gram (EPG), which is considered a high level of parasitism. All the 79 analysed samples were positive for strongylid-type eggs (100% prevalence). Cyathostominae were dominant, when compared to strongylinae and tricostrongylidae and with a total of 15 different morphological L3 types and/or species identified of the order Strongylida. Additionally, 27.8% were positive to *Parascaris* sp. and 2.5% contained *Oxyuris equi* in their expelled faeces. By the sedimentation method, we could only evidence a Trematoda egg. These results are consistent with the other studies performed in the same subspecies and represent the first survey of gastrointestinal parasites performed with this level of detail in the Pentezug Przewalski horse population.

Introduction

Przewalski horses (*Equus ferus przewalskii*) were introduced in the Hortobágy National Park (HNP), in 1997, with the main goal of managing the landscape in the Pentezug area of the HNP. It represented a good opportunity to study wild horses in a semiwild habitat, directly helping the conservation of this subspecies and other populations of *E. ferus* spp. Nowadays, the number of individuals is about 280, and besides the Przewalski horse, a herd of domestic cattle (*Bos primigenius taurus*), carefully bred to phenotypically resemble reconstructed aurochs (*Bos primigenius*), use this area for grazing (ZIMMERMAN et al., 2009). In equids, nematodes, when compared to cestodes or trematodes, are the group responsible for the greater diversity of parasites, which includes ascarids, as *Parascaris* sp., pinworms, as *Oxyuris equi* and many strongylids, all members of the superfamily Strongyloidea, except *Trichostrongylus axei*, from the Trichostrongyloidea (BOWMAN, 2009). Besides the species of parasites involved, the amount of parasites present is very relevant and defines if it represents an animal health and clinical problem for the individual or population. Equids, as a group of species, are more sensitive to parasites than, for example, sheep, goat or cattle, which leads us to consider faecal egg counts as lower than 500 Eggs per Gram (EPG) a low infection, 550 to 1000 EPG a moderate infection and more than 1000 EPG a high infection (SOULSBY, 1986; MADEIRA DE CARVALHO, 2006). Most of the studies performed in Przewalski horses under semi-natural conditions in other geographic regions are based in smaller populations and using an *in vivo* deworming method before the collection

(SLIVINKSA et al., 2006; KUZMINA et al., 2009, 2017), which was not done in this study, since the Pentezug population is managed without human interference or routine parasite control. Consequently, deworming is only performed in particular occasions, as the translocations of specific individuals. Regarding the described situation, the main goal of this study was performing a general but detailed survey of the gastrointestinal parasitology of the Pentezug population, as a tool for future parasite monitoring plans of these Przewalski horses.

Material and methods

Individual identification and sample collection

The animals were observed and identified, while defecating in a range of 50-100 meters. The majority of the observations were performed at the resting moments, while animals were eating and resting. Thereby, the identification was possible for most of the individuals due to the strong harem connections and proximity that defines the wild horses (RUBENSTEIN & HOHMANN, 1989). When an animal started defecating, the animals surrounding it were observed and the harem was determined and then, by using binoculars if necessary, it was possible to sex it and observe its body details, identifying the individual. However, most of the bachelors were not possible to be identified, since they do not establish the strong connections that we find inside the harem, which leads them to have irregular positions surrounding different harems. Afterwards, a small amount of fresh faeces was collected from the ground, using individual identified plastic bags and preserved at 4-5°C until reaching the laboratory. From the 79 samples collected, 62 were from identified harem members, 2 were from identified bachelors and 15 were from unknown bachelors.

Coprological methods

To quantify the EPG and evaluate the degree of parasite infection, we used the McMaster Technique. By mixing two grams of faeces with 28 ml of a saturated sucrose solution and filling a 0,30 ml Eggzamin® chamber, the total number of counted eggs inside the grids of both compartments is multiplied by 50 as a conversion factor, in order to obtain the EPG for each sample (MADEIRA DE CARVALHO, 2001). The Willis flotation and natural sedimentation methods, were performed afterwards in order to identify the light and heavy parasite eggs, respectively, present in the samples (LAJAS *et al.*, 2015). The Baermann method allows the migration and concentration of the L1 respiratory larvae present in a piece of faeces in the bottom of a conical cup full of water (LAJAS *et al.* (2015). Coprocultures were performed in order to obtain L3 infective strongylid larvae, which allowed the differentiation of parasites of this group, namely due to a recent dichotomic key and to the cyathostomins visual details recently described by SANTOS *et. al.* (2018). To have an idea of the proportion of infection by Strongylinae, Cyathostominae and Tricoststrongylidae, a total of 100 matured L3 larvae were counted and identified in each sample, in order to obtain the percentages of each mentioned family and subfamilies of the Order Strongylida.

Results and discussion

The average EPG for the whole 79 samples, was 1287 EPG (ranging from 250 to 5050), which is a high level of parasitism, according to the domestic horse values (SOULSBY, 1986; MADEIRA DE CARVALHO, 2006). KUZMINA et al. (2009, 2017) also reported high infection levels in other Przewalski horse populations. In reality, more than a half of the population (43/79) revealed a high infection level. In fact, 11 samples had less than 500 EPG, revealing a lower level of infection, 25 samples had between 550 and 1000 EPG, a medium level of infection and, finally, 43 samples showed more than 1000 EPG, which is considered a high level of infection, according to the domestic horse evaluation levels (SOULSBY, 1986; MADEIRA DE CARVALHO, 2006).

The different diagnoses found with coprology methods are illustrated in Fig.1. All the 79 analysed samples were positive for strongylid-type eggs (79/79) in the Willis floatation, the same happened with SLIVINSKA et al. (2006) and KUZMINA et al. (2009) in other Przewalski horse populations in different Ukraine regions.

From the total, 27.8% were positive to *Parascaris* sp. (22/79), which is higher than the one found by SLIVINSKA et al. (2006) and PAINER et al. (2011). In fact, Pentezug has a higher density of susceptible hosts when compared to densities presented in both previously mentioned studies, leading to a higher pasture contamination and increasing the probability of horse infection.

Globally, 2.5% (2/79) of the analysed wild horses contained *Oxyuris equi* in their expelled faeces. It is known that studies using mainly coprological methods and faecal samples, instead of anal scraping, may have some false negative results. Consequently, the described prevalence might be lower than the real one, due to the attachment of eggs and cementing fluid over the horse perianal area. However, with only faecal examinations, sometimes it is possible to find this parasite (BOWMAN, 2009). Using faecal samples, SLIVINSKA et al., (2006) reports infection by *O. equi* of 81% analysed Przewalski horses. On the other hand, other similar study in Russia only showed negative results for this parasite (KUZMINA et al., 2017). Consequently, with faecal exams, the results for *Oxyuris equi* are very variable. By the sedimentation method, we only could evidence a trematode egg. Since trematode infections in horses are considered to be extremely rare, we strongly suspect to be a case of pseudo-parasitism. This phenomenon mostly occur when the horses share a feeding area with cattle (NIELSEN & REINEMEYER, 2018), which, as mentioned above, is what happens in Pentezug.

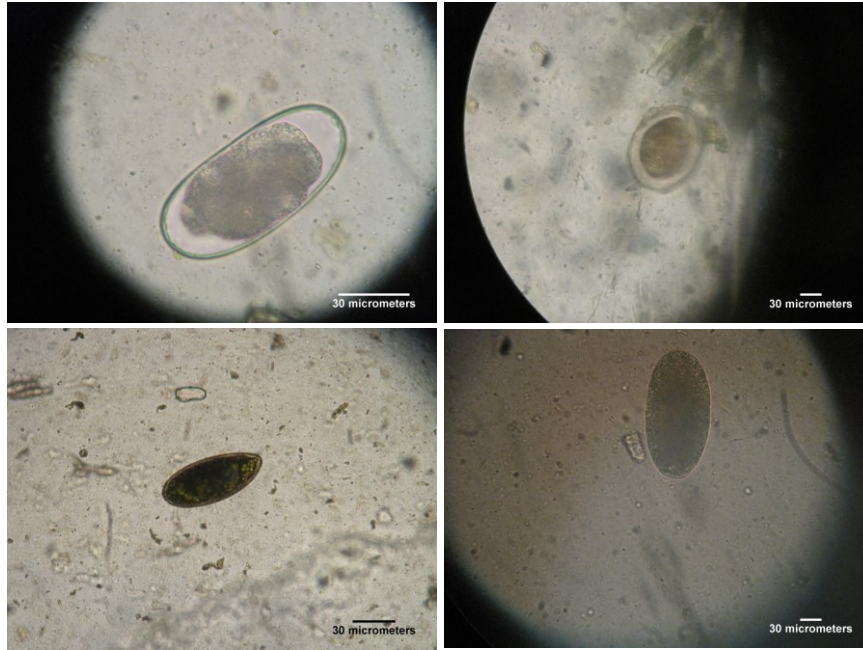


Fig. 1: Left to right, top row: strongyle-type egg, *Parascaris* sp. egg, Bottom row: *Oxyuris equi* egg, trematode egg. Originals.

The coprocultures showed that 100% of faecal samples had L3 and the strongylid infections were composed by an average of, approximately, 96.3% of Cyathostominae, 2.4 % Strongylinae and 1.3 % Tricostrongylidae, which is in accordance with the diversity of equid parasites in each one of these taxonomic groups. They also evidence parasite infections by multiple species, 15 different strongylids *sensu latum*, which usually happens in populations that are not usually dewormed (KUZMINA et al., 2016), as the considered population. KUZMINA et al. (2017) also revealed a dominance of Cyathostominae compared to Strongylinae, with no detection of Tricostrongylidae, which might be explained by the reduced size of the population tested by these authors when compared to this study, since our prevalence is low, but we found it. In our study, L3 of *Cyathostomum* s.l. type A (Fig.2-left) were the most frequent, occurring in 100% of the analysed samples. This agrees with a *post mortem* study, where three species producing this morpho-type (*Cylicostephanus minutus*, *Cyathostomum catinatum* and *Cylicocyclus nassatus*) had more than 90% prevalence, *C. minutus* with 100%, being the most frequent parasites found (SLIVINSKA et al., 2006). In the subfamily Strongylinae, *Strongylus vulgaris* (Fig.2-right) was the most common parasite of this group and the same happened with SLIVINSKA et al. (2006) and KUZMINA et al. (2009). This parasite species was found in 40.5% of the samples of our research, which is in the middle of the prevalence described in the mentioned studies. This prevalence should be considered as threatening, due to the potentially severe consequences of the larvae migration in the gastrointestinal arterial system, well described for the domestic horse. In fact, in the faecal samples found positive, at least one migration cycle occurred inside the host. *S.vulgaris* migrations can cause arteritis, thrombosis and/or infarction, leading to the sudden and unexpected death of an individual (NIELSEN & REINEMEYER, 2018).



Fig. 2: Two of the 15 different strongylid sensu lato found in our study: *Cyathostomum sensu lato* morpho-type A (left) and *Strongylus vulgaris* (right). Originals.

Even though some sampled individuals might be counted more than once, particularly the unknown bachelors group, the results are analysed and interpreted as populational results in the considered period of collection. As previously mentioned, this represents the most detailed parasitology survey performed at the Pentezug population, with a higher number of samples, when compared to similar studies performed in other European populations of Przewalski horses and consistent with those. In this way, it represents an idea of the parasite community of this population. However, a similar continuous monitoring, during the different seasons, can be a significative aspect for its management and possible reintroductions, considering the average 1287 EPG and maximal values around 5000 EPG. This future regular parasitological surveillance will enhance if there is a real influence of the parasitism in the body condition, reproduction or immune response of the host and in last instance, repercussions at the population level and survival rates, at both Pentezug and in future reintroduction sites.

Acknowledgements

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COXOFEMORAL LUXATION IN THE RED KANGAROO (*MACROPUS RUFUS*): THREE CASES

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Three female red kangaroos (*Macropus rufus*), aged 2 to 11 years, presented with traumatic coxofemoral joint luxation. Clinical signs included non-weight bearing lameness in two kangaroos, while the third kangaroo had significant lameness. The femoral head was craniodorsally displaced in two kangaroos, the most common presentation in domestic animals. In the third kangaroo, the femur was luxated caudodorsally.

Two kangaroos were treated within 48 h of injury with closed reduction under anaesthesia. Re-luxation occurred 24 h later in one case. Surgical reduction with a pin and toggle technique was attempted, however the kangaroo was humanely euthanized after intraarticular suture failure. Exercise restriction for two months was successful in stabilizing the affected hip in the second kangaroo. The third kangaroo's luxation was chronic when diagnosed, with pseudo-joint formation due at the injury. Medical records for this animal showed an episode of lameness five years prior, thought to be the original injury. Both surviving kangaroos were able to ambulate normally after recovery from injury.

Coxofemoral joint luxation is the most common type of luxation seen in small animal practice. Neither incidence rate nor treatment considerations for coxofemoral luxations have been reported in macropods. This type of luxation should be addressed immediately in kangaroos because of their bipedal mode of locomotion. Practitioners should be aware that lameness of varying degrees can be caused by coxofemoral luxation and that external signs of trauma are often absent in these cases. Closed reduction should be attempted first, with surgery reserved for cases of failure of conservative therapy.

REINTRODUCTION OF EUROPEAN BISON (*BISON BONASUS*) TO AUGUSTOWSKA FOREST (NORTHEASTERN POLAND) - AN EXAMPLE OF BEST PRACTICES

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European bison (*Bison bonasus*) is an endangered species, which was on the edge of extinction. Due to the restitution efforts, the population multiplied and it is necessary to find new areas, where the herds could be transferred. Augustowska Forest covers an area of about 115,000 ha. Together with the forests of Belarus and Latvia, it is one of the largest forest complexes in Europe, covering an area of ca. 250,000 hectares. The project of European bison reintroduction included 4 phases, of which the last one is still ongoing: data analysis, area preparation, creation and release of the new herd and its monitoring.

The process was time consuming and carried out under three conservation projects. It started in 2011 with an analysis of community attitude to the species and analysis of habitats to elaborate the target population size in the Augustowska Forest. The aim of preparatory stage was to enhance the natural conditions of the forest complex. This phase included mainly creation of 30 ha of mid-forest meadows and construction of 9 watering places. The forming of a new herd was preceded by the construction of an acclimatization enclosure with appropriate conditions in 2017. The herd consisting of 7 selected individuals was formed and released in 2018. Since the release, a complex monitoring, including genetic, veterinary, spatial and ecological, is being conducted. The report shows that the restitution of European bison is challenging, but it is successfully conducted and should be continued in the future.

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INTRAMUSCULAR ADMINISTRATION OF ALFAXALONE FOR ANAESTHESIA IN GOLDEN-HEADED LION TAMARINS (*LEONTOPITHECUS CHRYSOMELAS*)

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Alfaxalone is a neurosteroid anaesthetic commonly used in small animal and exotic pet practice (JONES, 2012). This study aimed to evaluate intramuscular alfaxalone (Alfaxan®, Jurox Inc., USA) doses for general anaesthesia in the endangered golden-headed lion tamarin (GHLT) (*Leontopithecus chrysomelas*) (KIERULFF et al., 2008). A randomised clinical trial was performed on 15 healthy, male, subadult and adult GHLT undergoing vasectomy to compare the effects of alfaxalone at three single intramuscular doses (6, 10 and 12 mg/kg). After immobilisation, animals received infiltrative local anaesthesia with lidocaine (8 mg/kg) at the incision site. The mean duration of effect (for 6, 10 and 12 mg/kg, respectively: 30.7 [95% CI [27.6-33.8]], 37.2 [34.3-40.1] and 47.2 [36.6-57.8] minutes) was significantly ($p=0.002$) longer at the 12 mg/kg, then at 6 mg/kg dose. Mean induction time (2.2 [1.8-2.5], 2.2 [1.0-3.5], and 1.6 [0.8-2.4] minutes) and recovery time (42.5 [28.6-56.4], 67.5 [54-81] and 72.2 [43-101.4] minutes) did not present a significant statistical difference between groups. Neither did overall mean heart rate (331.0 [322.3-339.7], 337.7 [331.7-343.6] and 313.3 [306.1-320.6] beats per minute), respiratory rate (32.8 [31.1-34.6], 37.3 [34.9-39.7] and 33.8 [32.1-35.5] breaths per minute), mean blood pressure (127.8 [123.4-132.1], 135.4 [130.0-140.8] and 129 [125.6-132.4] mmHg) and temperature (37°C [36.8-37.1], 36.95°C [36.7-37.2] and 36.63°C [36.5-36.8]). This is the first report of the use of alfaxalone in GHLT; it appears safe and provides a short and cardiovascular stable anaesthesia. Muscle tremors were noted during induction and recovery. Future studies should evaluate alfaxalone in combination with other drugs for enhanced muscle relaxation.

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**MONITORING OF *TOXOPLASMA GONDII*, *NEOSPORA CANINUM*,
ENCEPHALITOZOOM CUNICULI, *CHLAMYDIA ABORTUS* AND *COXIELLA*
BURNETII IN ZOO LJUBLJANA, SLOVENIA**

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Problems with parasitic and bacterial infections and their interspecies transmissions are common in zoological gardens and could pose serious health damage to captive animals. The aim of the study was to detect presence of selected parasites and bacteria in animals from Ljubljana ZOO, Slovenia. Antibodies to *Toxoplasma gondii*, *Neospora caninum* and *Encephalitozoon cuniculi* were found in 37 %, 3 % and 55 % of tested mammals (n = 123) and in 0 %, 3 % and 34 % of tested birds (n = 65), respectively. There was statistical significant difference of *T. gondii* prevalence in Carnivora compared to Cetartiodactyla (p = 0.0227) and Primates (p = 0.0084). Herbivores had higher probability to be infected with *E. cuniculi* compared to omnivores (p = 0.0015). Sera of 39 wild rodents trapped in zoo were negative for all three tested parasites. Antibodies to *Chlamydia abortus* and *Coxiella burnetii* were not detected in any of zoo animals. *T. gondii* was detected by PCR in blood sample of two mute swans (*Cygnus olor*), one laboratory mouse (*Mus musculus* var. *alba*), two eastern house mice (*Mus musculus*), one yellow-necked field mouse (*Apodemus flavicollis*) and one striped field mouse (*Apodemus agrarius*). Positive samples were genotyped by a 15 microsatellite markers method in a single multiplex PCR assay and one sample from a mute swan was successfully characterized as *T. gondii* type II. This study is the first comprehensive report including seroepidemiology and molecular detection of selected pathogens in zoo animals from Slovenia, however further research is needed.

VAGINAL FOREIGN BODIES AS A WARNING SIGN OF UTERINE OR URINARY TRACT DISEASES IN JAPANESE MACAQUES (*MACACA FUSCATA*)

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Four female Japanese macaques (*Macaca fuscata*) from the same group presented with vaginal foreign bodies associated with diseases of the reproductive (n=3) or the urinary (n=1) tracts over a 2-year period. In all cases, foreign bodies were accompanied by a mild to marked diffuse vaginitis. Associated anomalies consisted of a cavernous uterine hemangioma in a 20-year old individual, a diffuse endometritis in a 21-year old individual, an endometrial *in situ* carcinoma in a 24-year old individual, and a chronic cystitis in a 24-year old individual. Vaginal foreign bodies were not reported in any other females from the same group over the 2-year period. Vaginal foreign bodies are occasionally reported in primates in biomedical research, but do not appear to be related to urogenital conditions or to have a significant impact on reproductive success (FORD et al., 1988). Pain in the caudal abdomen or a change in the vaginal flora may be responsible for the introduction of foreign bodies into the vagina in children (CHINAWA et al., 2013). Whether this abnormal behavior is innate in this primate species or exclusive to this captive population remains to be clarified. However, as reproductive diseases are more prevalent in ageing females of this species (GALL et al., 2018), attention should be paid to the introduction of foreign bodies in the vagina of Japanese macaques under human care. Finally, this case series emphasizes the recommendation that abnormal behaviors should be investigated for underlying medical conditions.

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EVALUATION OF A BUTORPHANOL, DETOMIDINE AND MIDAZOLAM COMBINATION FOR IMMOBILISATION OF CAPTIVE SCIMITAR-HORNED ORYX (*ORYX DAMMAH*)

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The aim of this study was to evaluate the effects of a combination of butorphanol, detomidine and midazolam (BDM) and its partial reversibility in scimitar-horned oryx (SHO). This combination of drugs has been previously used Nile Lechwe (LARICCHIUTA et al. 2012)

Sixteen captive SHOs (9 female and 7 male), weighting 117.5 ± 35.49 kg and 5.47 ± 2.11 years old, were immobilized for routine clinical examination, with butorphanol (Alvegesic 10 mg/ml®, Alvetra Ag, Italy) 0.11 ± 0.01 mg kg⁻¹, detomidine (Sedaquick 10 mg/ml® Fatro, Italy) 0.2 ± 0.001 mg kg⁻¹ and midazolam (Midazolam Ibi 5 mg/ml®, I.B.I., Italy) 0.05 ± 0.001 mg kg⁻¹ administered intramuscularly (IM) with a blowpipe. Upon recumbency (T0), physiological parameters and anaesthetic depth were monitored each 10 minutes (T10, T20 and T30). An arterial blood sample was collected at T20. At the end of the procedures, immobilization was partially reversed with atipamezole (Antisedan®, Orion, Italy) 0.26 ± 0.02 mg kg⁻¹ IM. Quality of induction, immobilization and recovery were scored [scored 1 (excellent) to 4 (poor)].

The lateral recumbency was rapidly reached in 10.1 ± 3.5 minutes and the median induction score was 1 (range 1–3). Heart rate varied from 30 to 69 beats minute⁻¹, respiratory rate from 12 to 68 breaths minute⁻¹ and temperature from 36.9 to 39.6 °C. Gas-exchange assessment revealed: pH (7.46 ± 0.07), PaCO₂ (42.23 ± 5.06 mmHg), PaO₂ (78.89 ± 13.9 mmHg) and SaO₂ (94.738 ± 4.7 mmHg). Median recovery score was 1 (range 1–2) and the animals were walking within 14.66 ± 3.7 minutes after partial reversal.

The drug combination proved to be effective in immobilizing captive healthy SHOs with minimal cardio-respiratory changes and with the avoidance of ultra-potent opioids. The dosages of the drugs were lower than the ones previously used in the Nile Lechwe.

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A CASE OF PORENCEPHALY IN A SIAMANG (*SYMPHALANGUS SYNDACTYLUS*)

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A four-year-old female Siamang (*Symphalangus syndactylus*) was presented to a veterinary neurologist one week prior to euthanasia of the animal and subsequent necropsy following a 2-month history of blindness and progressive mental deterioration. On neurologic examination, the siamang showed dull mentation, but no signs of ataxia or impaired gait.

Magnetic resonance imaging (MRI) revealed a homogenous T2-hyperintense, non-contrast enhancing lesion within the mesencephalic duct with secondary obstructive hydrocephalus, characterized by distension of the lateral and third ventricles with compression of the optic chiasm. No evidence of a space-consuming mass was found.

At necropsy, the brain was mildly oedematous and there was bilateral thinning of the cerebral cortex with distension of the lateral ventricles. Serial sectioning revealed a bilateral, asymmetric, smooth walled, cleft-like cavitation filled with clear fluid effacing the frontal lobe, with deviation of the midline to the right, reaching the thalamus and, more caudally, the dentate gyrus of the hippocampus.

Microscopic lesions were overall unremarkable, ranging from diffuse leukomalacia and cystic lesions lined by a layer of flattened glia to moderate atrophy of the hippocampus. Our morphologic diagnosis was a bilateral and asymmetric porencephaly.

This rare pathology has been related to a destructive process in prenatal life (MAXIE et al., 2016). Developmental porencephaly is based on a neuronal migration disorder in the developing cerebral hemisphere, while encephaloclastic porencephaly is associated with cerebral injury resulting from ischemia, toxicity, trauma, and infection (usually virus) during the fetal stage (HORI et al., 2015; HIROWATARI et al., 2012).

To our knowledge, this is the first report of porencephaly in a siamang.

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FIRST REPRODUCTION SOUNDNESS EVALUATION OF CAPTIVE ASIATIC CHEETAH (*ACINONYX JUBATUS VENATICUS*) IN IRAN

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The Asiatic cheetah is one of three cheetah subspecies and is classified as critically endangered. Today, only 30 individuals may have survived and only occur in Iran.

Since 2015, a captive breeding project was established at Pardisan Park in Tehran, and currently 1,2 Asiatic cheetahs are kept, at the age of 10, 8 and 2, respectively.

The older female was bred in February 2016, but aborted at day 45 after mating. No further breeding activities occurred ever since. Since reproducing Asiatic cheetahs is of utmost importance for survival of this subspecies, in 2017 and 2019 full reproductive assessments were undertaken, including fecal estradiol, ultrasonography and semen collection and preservation. Despite a phase of acyclicity in 2017 for the older female, both animals showed active ovaries with 5 and 3 antral follicles of 0.4 and 0.5 cm, respectively. The older female had a more diffuse echogenicity of the uterus, which may hint towards beginning endometrial hyperplasia.

Semen quality in 2017 and 2019, respectively (volume: 300 and 240 µl, total motility: 80 and 30%, sperm concentration: 32 and 18 × 10⁶/ml, morphology: 10 and 28% normal sperm), was within the range of reports on African cheetah and thus the male was classified as fertile. Due to a lack of sexual interest from the male, it was decided to opt for artificial insemination.

Here we report results of male and female reproductive assessments and a first AI trial in the Asiatic cheetah.

SEMEN CRYOPRESERVATION IN AMERICAN FLAMINGO (*PHOENICOPTERUS RUBER*) WITH DIFFERENT PERMEATING CRYOPROTECTANTS

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The American flamingo is a useful model for development of successful semen cryopreservation procedures to be applied to threatened related species from the family Phoenicopteridae, and to permit banking of genetic material. The present study sought to develop effective cryopreservation protocols through examining the influences of two permeating cryoprotectants. Semen samples were collected from 21 American flamingos, and diluted with a glutamate-polyvinylpyrrolidone-based medium. DMA (6%) or Me2SO (8%) was then added, and the samples allowed to equilibrate for 10 min at 5°C before loading into 0.25 mL straws; finally sperm cryopreserved using a two-step cooling method: from 5°C to -35°C at 7°C/min, and then from -35°C to -140°C/min at 60°C/min. Frozen samples were thawed at 5°C and assessed for motility variables by CASA. Propidium iodide and SYBR-14 were used for the examination of membrane integrity. DNA fragmentation was assessed by TUNEL. No differences were seen in the values of frozen-thawed sperm variables between DMA and Me2SO for total motility (14.9 vs 17.4%), straight-line velocity (22.1 vs 22.2 µm/s), curvilinear velocity (29.4 vs 33.2 µm/s), average path velocity (24.8 vs 25.9 µm/s), sperm viability (48.2 vs 43.6%) and TUNEL+ sperm (16.5 vs 17.2%). The findings demonstrated that both DMA and Me2SO were equally suitable as cryoprotectants for the cryopreservation of flamingo sperm.

AMPUTATION OF THE RIGHT OUTER PEDIPALP OF A GIANT FOREST SCORPION (*HETEROMETRUS SPINIFER*) DUE TO INFECTION

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A Giant Forest Scorpion (*Heterometrus spinifer*) was submitted to the University of Hannover Veterinary Clinic. Three weeks prior to submission, there was an injury resulting in a defect of the manus and tarsus of the right pedipalp. One week prior to submission, the owner detected what appeared to be a small maggot protruding from an ulcerated lesion of the right pincer. Myiasis and possible infection were considered. Additionally, the owner reported a foul smell emanating from the scorpion. Clinical examination revealed that, except for the manus and tarsus of the right pincer, the scorpion exhibited normal defensive behavior and was otherwise normal in appearance. However, a foul smell was detected. The scorpion was only capable of lifting the left pincer; the right pincer could only be slightly moved and lifted. There was severe necrosis of the right manus and a foul odor emitting from this body part. Therefore, a decision was made to amputate the right manus and tarsus to prevent the development of an ascending infection that may lead to death. The scorpion was placed in a box and anesthesia was carried out in a box flooded with isoflurane. Local anesthesia was also used and consisted of multiple drops of proparacaine hydrochloride (ophthalmic solution 0.5%) applied at the surgical incision at the right trochanter. Loss of hemolymph was prevented using tissue glue applied on the surgical site. Recovery occurred within 5 minutes after the end of the surgery and was uneventful.

CHARACTERISATION OF THE FAECAL MICROBIOME OF A CAPTIVE POPULATION OF CALLITRICHIDS USING NEXT-GENERATION SEQUENCING

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The gastrointestinal microbiome is increasingly recognised to be a key determinant of many host physiological processes, and an important factor in a range of disease states. Marmoset Wasting Syndrome (MWS) is a common cause of morbidity and mortality in captive Callitrichidae, yet its epidemiology and pathogenesis remain incompletely understood. Investigating the microbiome of captive Callitrichidae in health and disease may lead to a better understanding of MWS. We utilised next-generation sequencing to characterise the faecal microbiome of captive *Cebuella pygmaea* (n = 5), *Saguinus oedipus* (n=4), *S. imperator* (n=3) and *Leontopithecus rosalia* (n=3) at Adelaide Zoo, South Australia. Diets consisted of raw vegetables and leafy greens, Primate Meal (Wombaroo-Passwell, Glen-Osmond, South Australia) made into a 'cake', invertebrates, and a high-protein component (variably kibble, egg, yogurt etc); fruit-free diets had been in place for approximately 12 months prior to sampling. All animals were considered healthy except for one *C. pygmaea* showing weight loss and hair loss, both features of MWS (this animal subsequently improved spontaneously). MWS, refractory to treatment, was the cause of death/euthanasia in four *C. pygmaea* at Adelaide Zoo in preceding years. Fresh faecal samples (n=3-6 per individual) were collected following observed defecation over several weeks. To achieve adequate DNA concentration, equal parts of all samples from each individual were pooled for analysis. *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, and *Actinobacteria* were the most abundant phyla, constituting 98% of bacterial diversity. The *S. oedipus* had a more diverse microbiome than the other species; this may be associated with their larger, more complex, mixed species (co-housed with conures) enclosure. Previous studies on non-human primates (NHPs), reviewed by CLAYTON et al. (2018), suggest that captivity is associated with reduced diversity and altered composition of the microbiome compared with wild conspecifics, and that the microbiome of captive NHPs includes microbial taxa that dominate the microbiome of modern humans. The results of the present study are consistent with the latter finding; data on free-ranging Callitrichidae are required to investigate the former. These characteristics of the captive callitrichid microbiome could be a factor in the development of MWS. There were no statistically significant differences between the four healthy *C. pygmaea* and the individual with MWS-consistent clinical signs. This could be a result of the small sample size, though given the high incidence of MWS in *C. pygmaea* at Adelaide Zoo, the possibility that all animals in the group harbour a microbiome predisposing to MWS is considered.

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THE ILLEGAL USE OF SNARES AND GIN TRAPS THREATENS ENDANGERED PERSIAN LEOPARDS (*PANTHERA PARDUS SAXICOLOR*) IN IRAN

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The present report documents nine cases in the course of the last 7 years of leopards being caught accidentally in snares or gin traps that were set by local people against wild boars (*Sus scrofa*) or less frequently jungle cat (*Felis chaus*). It details the type and diversity of lesions resulting from these trapping methods, and informs a broader audience about the newly reported threat posed by snares and gin traps to leopards in Iran. Three of these leopards died as result of severe injuries, two of them were rescued and survived albeit disabled and one animal could be released to the wild. These cases reveal the growing problem of habitat disturbance and loss for leopards and their natural prey in the Alborz mountain range and Caspian lowlands of Iran. Before this study a review of 71 mortality cases throughout the country between 2007-2017, found that 70% of documented mortality resulted from intentional killing and poisoning, followed by road accidents (18%). Snares and gin traps caught Persian leopards by different parts of their bodies and with variable effects. The indiscriminate accidental trapping of leopards in northern Iran is probably underestimated and because of its associated mortality level, it likely affects the long-term viability of the leopard population by removing mature individuals of both sexes from a population already assessed to be declining. There is an urgent need to stop the widespread use in Iran of snares and gin traps as a control method of wild boar populations, which also affects other mammals from small Mustelids to large Ursids.

VIRAL SEROCONVERSIONS IN A CLOSED BREEDING GROUP OF OLD WORLD MACAQUES

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The aim was to establish a specific pathogen-free (*SPF*) breeding colony of Indian/Burmese rhesus macaques (*Macaca mulatta*) and cynomolgus macaques (*Macaca fascicularis*) by using ELISA-, virus isolation- and PCR techniques. A combination of three consecutive negative ELISA- and virus isolation results in the first three months of screening was used to establish the first *SPF* breeding groups. The status of these groups was initially checked twice every year, and when no positive animals were detected, screening was restricted to once a year. Animals with equivocal or positive results were taken from the *SPF* breeding groups and housed in the non-*SPF* breeding groups.

The research was (and is) mainly focussed on the macaque alpha herpes virus (B-virus), simian T-cell lymphotropic virus (STLV), and simian retrovirus type D (SRV). Testing for SIV was aborted after some years due to the fact that all animals were negative for SIV for several years. When we started screening in 1994, 48% (n=258) of the colony of rhesus macaques was negative for all three viruses. The remaining animals were infected with one, or a combination of the three viruses mentioned above. During the last evaluation in 2015, 83% of the macaques (864/1039) revealed the *SPF* status and hopefully the results of the 2018/2019 evaluation will reveal again a rise numbers of *SPF* macaques.

Hendrik Niphuis (22-10-1959/Netherlands) finished Zoology-HLO, in Utrecht in 1985 and started working as junior research assistant at the Cancer Centre Rotterdam (1985-1987) and TNO, Rijswijk (1987-1995) involved in AIDS-/Hepatitis-/Herpes viruses research. He is now senior research assistant at the BPRC Rijswijk (1995- now) at the dept. virology and fulltime working for Primate Viral Diagnostics, screening monkey populations from all over the world.

STRESS-HAEMOCONCENTRATION DURING THE CAPTURE AND TRANSPORT OF FREE-RANGING WHITE RHINOCEROSSES (*CERATOTHERIUM SIMUM*) SEDATED WITH EITHER AZAPERONE OR MIDAZOLAM

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Haemoconcentration indicates an increased ratio of red blood cells and large molecules (>69 kDa) to the plasma volume and has been associated with acute stress in laboratory animals. Here, we measured the response of common indicators of haemoconcentration (erythrocyte physical variables and serum proteins) to chemical-capture and transport in twenty-three free-ranging sub-adult white rhinoceros bulls sedated with either azaperone or midazolam. Rhinoceroses were captured from helicopter with a combination of etorphine (3-4 mg, i.m.; Captivon®, Wildlife Pharmaceuticals, 9.8 mg/mL) plus either azaperone (n=11, i.m.; Zaprnil®, Wildlife Pharmaceuticals, 50 mg/mL) or midazolam (n=12, i.m.; Dazonil®, Wildlife Pharmaceuticals, 50 mg/mL) at five times the etorphine dose, mg. Once immobilised, an intravenous catheter was placed in an auricular vein and butorphanol (15-25 mg; i.v.; Wildlife Pharmaceuticals, 50 mg/mL) was administered to allow for loading of the rhinoceros, followed by an intravenous bolus of diprenorphine (9-15 mg, i.v.; Activon®, Wildlife Pharmaceuticals, 12 mg/mL) to reverse the immobilisation. Additional azaperone or midazolam, respectively, were administered at 25 times the etorphine dose, mg, every two hours during transport, and serial blood samples were collected from the intravenous catheter at (T1) capture; (T2) start of transport; and (T3) after six hours of transport. Changes in measured variables over time and between groups were compared using general mixed effects models.

Packed cell volume, red blood cell count, and haemoglobin concentrations were highest at T1 and decreased from T1 to T3 indicating haemoconcentration during capture likely resulting from the effects of stress-induced catecholamine-release (i.e. hypertension and splenic contraction). Mean corpuscular haemoglobin concentration (MCHC) and red blood cell distribution width (RDW) were higher at T1 compared to T2, but did not change from T2 to T3, probably reflecting red cell changes in response to alterations in plasma osmolarity. Concentrations of albumin and globulin were highest at T1; albumin concentrations tended to be higher in midazolam-rhinoceroses possibly reflecting differences in the cardiovascular effects of the drugs, or acid-base-equilibria. Globulin concentrations were significantly lower in midazolam-rhinoceroses likely indicating an immunosuppressive effect of the benzodiazepine. The results indicate that capture of white rhinoceroses is associated with acute stress. Better understanding the clinical relevance of the rhinoceroses response to the sedative drugs administered during transport is critical as it may play a role in the development of disease and translocation failure.

METASTATIC OSTEOSARCOMA IN A FIVE YEAR OLD BARBARY LION (*PANTHERA LEO LEO*)

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In domestic animals, osteosarcomas are the most common primary neoplasms of the skeleton. In cats and dogs it commonly involves the appendicular rather than the axial skeleton, and haematogenous metastases to other organs are very common. Reports of this neoplasm in lions are scarce. Here, we report a case of a highly malignant, non-productive osteoblastic osteosarcoma in a five-year-old male Barbary lion (*Panthera leo leo*). The lion showed weakness in his hind legs, as well as neurological symptoms (e.g. head tilt, staggering). Due to worsening of the symptoms, he was euthanized. The first thing noted on necropsy were several, centrally indented, rather solid, macroscopically well circumscribed masses in the liver of up to approximately 5 cm in diameter, several flat white masses in the lungs, one flat approximately 2 cm x 2 cm large mass in the left ventricle of the heart and several pea-sized masses in the mediastinum. Due to the clinical symptoms, the brain and spinal cord were examined. Highly altered bone structures were noted on the skull and the atlas. The foramen ovale as well as the left wing of the atlas were shattered and the vertebral body was extensively infiltrated by neoplastic tissue, as was the surrounding muscle and draining lymph nodes. The os occipitale and the caudal area of the os parietale showed a highly irregular and roughened surface. Parts of the brain meninges were thickened and opaque. In histology the bone masses proved to be a highly cellular, highly malignant non-productive osteoblastic osteosarcoma with poorly differentiated metastases in lung, heart, liver and muscle. To the authors' knowledge, this is the first report of a metastatic osteoblastic osteosarcoma in a lion, and highlights again the importance of a thorough pathological and pathohistological analysis.

LONGTERM REPRODUCTIVE MONITORING OF A CAPTIVE MALE HARP SEAL (*PAGOPHILUS GROENLANDICUS*) AND EVALUATION OF DESLORELIN IMPLANT CONTRACEPTIVE EFFECT

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Over a four year period, 12 serial transcutaneous ultrasound exams of testis and prostate were conducted on a 17 year old captive born male harp seal with no access to female from the same species during the time of the study. All exams were conducted in behavioral restraint. Harp seals are seasonal breeders with a breeding period occurring in late winter to early spring. During each exam, testis and prostate size and echogenicity were recorded. Serum testosterone levels were measured during the breeding season in year three and four of the study in conjunction with two ultrasound exams. Considering the elliptic shape of the prostate on a longitudinal section, prostate size was determined by measuring short and long axis of longitudinal section and calculating the area of the ellipse. A significant increase in size and echogenicity of the prostate was observed during the breeding period. Prostate area was $39.43 \pm 9.3 \text{ cm}^2$ in february-march and $20.21 \pm 6 \text{ cm}^2$ outside this period. In year three of the study, a 9.4 mg deslorelin acetate implant was inserted subcutaneously mid-february at the onset of the breeding period while prostate area was 39.56 cm^2 . Four weeks after the implant insertion, a decrease in prostate size to 16.7 cm^2 has been observed along with a decrease in echogenicity. Twelve months after implant insertion, prostate size and serum testosterone levels were in the same range as the years previous implant insertion. This study suggests that 9.4mg deslorelin acetate implant effects on reproductive activity is unlikely to last for two consecutive breeding periods.

DIAGNOSIS AND SURGICAL MANAGEMENT OF CHOLELITHIASIS IN AN ADULT BEARDED DRAGON (*POGONA VITTICEPS*)

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A 5-year-old male bearded dragon (*Pogona vitticeps*) was presented with a 4-day history of anorexia after 2 weeks of dysorexia. Husbandry was adequate, and the diet was composed of calcium/multivitamins supplemented arugula salad (80%), mixed vegetables (15%) and thawed crickets (5%). A firm, spherical and mobile 2-cm in diameter mass was palpated in the cranial coelom. Glycemia was slightly increased but remained below levels described in cases of somatostatinomas in bearded dragons. Radiographs were unremarkable but ultrasonographic evaluation was highly suggestive of cholelithiasis. A markedly enlarged gallbladder containing a gallstone was detected on coeliotomy. Total cholecystectomy was accomplished by gentle dissection of the liver lobe adjacent to the gallbladder, double ligation followed by full resection of the gallbladder. Post-operative oral treatment included meloxicam 0.2 mg/kg q24h x5d, tramadol 11 mg/kg q24h and amoxicillin-clavulanic acid 20 mg/kg q12h x7d. The bearded dragon resumed eating after seven days. Gall bladder wall was unremarkable on histology. No stone composition analysis was performed. Although cholelithiasis has been described previously in bearded dragons, the management and prognosis of this condition are poorly described. Cholelithiasis might be underdiagnosed and should be included in the differential diagnosis of cranial coelomic masses. Cholecystectomy resulted in a positive outcome in the present case. Arugula salad contains glucosinolates contributing to the synthesis of thiocyanates, and could therefore favor secondary nutritional hypothyroidism, which is a contributing factor of cholelithiasis in people. Thus, diversification of vegetables in bearded dragons' diet should be encouraged pending more research on cholelithiasis aetiology in this species.

HERPESVIRUSES IN SELECTED SPECIES OF FREE-RANGING BIRDS OF PREY IN SLOVENIA

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Herpesviruses (HVs) affect many mammalian and avian hosts. They cause an inclusion body disease or herpesvirus hepatitis in falcons, owls, and eagles, respectively (GAILBREATH and OAKS, 2008). Moreover, recently, we reported the detection of novel HVs in free-ranging owls in Slovenia (ŽLABRAVEC et al., 2018). The aim of the study was to further investigate HVs in selected species of free-ranging birds of prey in Slovenia. Oropharyngeal and cloacal swabs were collected in 54 birds of prey from seven different species: Common Buzzard (*Buteo buteo*, N=27), Common Kestrel (*Falco tinnunculus*, N=16), Eurasian Sparrowhawk (*Accipiter nisus*, N=7), European Honey Buzzard (*Pernis apivorus*, N=2), Northern Goshawk (*Accipiter gentilis*, N=1), and Eurasian Hobby (*Falco subbuteo*, N=1). Birds were presented to Clinic for Birds, Small Mammals and Reptiles, Veterinary faculty as wildlife casualties from various location in Slovenia between October 2017 and January 2019. For the detection of HVs, a nested PCR that targets a region of the HV DNA polymerase gene was used (VANDEVANter et al., 1996). HVs were detected in oropharyngeal swabs of 7 out of 54 birds. Six HV sequences detected in Common Buzzards were almost identical to novel HV sequences detected in Common Buzzard in Hungary. HV sequence detected in Common Kestrel shared 87% nt identity to psittacid herpesvirus 1. Our study showed that two diverse HVs that are related to avian alphaherpesviruses were detected in investigated birds of prey. Furthermore, they are also different from recently reported HVs from free-ranging owls in Slovenia (ŽLABRAVEC et al., 2018).

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SPREADING OF *MACRORHABDUS ORNITHOGASTER* IN ORNAMENTAL BIRD AVIARIES IN PIEDMONT

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Avian gastric yeast (AGY) *Macrorhabdus ornithogaster*, has a worldwide distribution and currently colonize the proventriculus and ventriculus of a wide of bird species (PHALEN, 2014), representing a potential threat to bird breeders. The aim of this study was to assess the presence of AGY in breeding ornamental birds, rearing in Piedmont. The study was conducted in 15 breeding centers of ornamental birds and on birds from 3 private owners. Pool of fecal samples were examined by microscopic test for the presence of yeast. Level of shedding of AGY was evaluated using a semi-quantitative scale (LANZAROT et al., 2013). On some dead animals was performed histological examination. The presence of AGY was observed in 12 of the 18 farms (67%): 61% in commercial farms and 50% in private ones. The prevalence was 61%, in farms with 1 species, while in multispecies farms the prevalence was 44%. A high number of fecal parasitic and fungal co-infections (62%) was observed. From dead animals, the histological exam confirmed AGY infection. The study confirms that *M. ornithogaster* is widespread: 2/3 of farms and more than half of the sampled cages harbored AGY. Moreover, the parasitic and fungal co-infections do not seem to influence the risk of infection for AGY ($P=0.759$).

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AN ABNORMAL SERIES OF SPATIO-TEMPORAL STRANDINGS IN ADULT FEMALE HARBOUR SEALS (*PHOCA VITULINA*) IN THE GERMAN NORTH SEA

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From December 4th, 2018, to January 13th, 2019, 11 adult female harbour seals (*Phoca vitulina*) in good nutritional status stranded dead along the coastline of Büsum, Schleswig-Holstein, Germany. All animals underwent necropsies following a standardized protocol. Samples of all animals were sent for histology, microbiology and virology analysis (data of four animals are still pending). The females were either pregnant, had aborted or were suspected to have aborted recently. Six females showed up to 10 cm long lacerations in either the vagina, the uterus or both. In one case, a foetus was found in the abdomen. In another, bone and cartilage fragments indicating a macerated foetus were present in the abdomen. Analyses of virology samples, including PDV, PHV and influenza, were negative. Histology revealed moderate to severe hyperaemia in the lungs and central nervous system of all animals, as well as severe inflammatory processes associated with the genital tract lacerations. Moderate to severe infection with either one or a combination of *Escherichia coli* and beta-haemolytic streptococci, including *Streptococcus phocae*, was present in the lungs, kidneys, livers, spleens, intestines and the genital tract. Based on those findings, it is likely that the animals suffered from acute septicaemia and died due to septic shock. It is noticeable that only apparently healthy, adult females were affected. This may indicate that the gender and/or reproductive status plays a major role in these cases. The aetiology of the lacerations in the genital tract could not be determined so far.

SUSCEPTIBILITY OF ZOO ANIMALS TO *MYCOBACTERIUM AVIUM* SUBSPECIES *PARATUBERCULOSIS*: A REVIEW OF PUBLISHED REPORTS

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Mycobacterium avium subspecies *paratuberculosis* (MAP) is the causative agent of Johne's disease (JD), a contagious, chronic and typically fatal enteric disease that preferentially affects ruminants, but has also been described in several non-ruminant species.

This study attempts to summarize information on the association of MAP infection and induced disease in zoo animals. Published reports concerning the detection of MAP and the transmission and epidemiology in zoological gardens were reviewed. Based on the presence of clinical symptoms and associated diagnostic methods (pathological and histopathological examinations, serology, cultivation or molecular biology of feces or tissues), cases could be categorized by consistent terminologies and case definitions (exposed, infected, diseased) and susceptible families were identified.

Infection and resulting disease with typical granulomatous and acid-fast positive lesions in the intestines are commonly reported in bovids, cervids and camelids, although diagnosis can be difficult in early stages of pathogenesis. Single reports of clinical JD are also documented in Equidae and Cercopithecidae. The diagnosis of MAP infection by culture or PCR from tissue samples is described in few reports for Giraffidae, Suidae, Tapiridae, Callitrichidae and Procaviidae. Positive serological tests or detection of MAP from fecal samples of several other species suggest exposure but should always be interpreted with caution, as both methods are insufficient to confirm infection.

Involving this anthology in the verification of JD as a differential diagnosis, once chronic emaciation or diarrhea are observed within a collection, can be crucial for the interpretation and classification of positive-tested animals and the resulting consequences in disease management.

SEVERE TAPEWORM INFESTATION ASSOCIATED AND ASSOCIATED HISTOPATHOLOGICAL LESIONS IN A PURPLE-THROATED CARIB (*EULAMPIS JUGULARIS*)

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The Purple-throated Carib (*Eulampis jugularis*) is an endemic hummingbird of the Lesser Antilles. Very few data are available on diseases of this bird. A wild-caught female Purple-throated Carib, kept in an Italian scientific center, was presented for necropsy after death. Although gross lesions were not observed, a large number of tapeworms showing an unarmed scolex was evidenced in the small intestine. Histologically, this severe tapeworm infestation was associated with severe mucosal hyperplasia, submucosal gland proliferation and severe lymphoid follicular hyperplasia, especially in the duodenum. Consequent to the strong thickening of the intestinal mucosa, areas of intestinal sub-obstruction, where the hyperplastic mucosa filled the entire duodenal cross-section, were evidenced. Based on parasite and egg morphology and PCR analysis, the isolated unarmed tapeworm species was identified as belonging to the family Hymenolepididae. Although tapeworm infestations have been reported in hummingbirds (Widmer et al., 2013), no data on prevalence of Hymenolepididae infestations in captive or free-ranging birds are available and no associated lesions have been described in previous studies. This report is the first description of pathological lesions and death associated with tapeworm infection in a hummingbird.

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ASSESSMENT OF CARDIAC FUNCTION IN A FAMILY OF FOUR SNOW LEOPARDS (*PANTHERA UNCIA*)

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This study presents the results of the transthoracic echocardiographic evaluation of a family of 4 clinically healthy snow leopards (*Panthera uncia*) after the death of one of their 7-month-old cubs. The animal presented a rapidly evolving respiratory distress that worsened until death. Necropsy revealed a congenital cardiomyopathy consisting in a left atrio-ventricular communication confirmed by histologic examination. Because of the frequent genetic origin of cardiomyopathies in large felids, an assessment of cardiac function of the parents and two other cubs of the family was decided. Transthoracic echocardiography was performed under general anaesthesia with butorphanol (Torphasol, Axience, France, 0.17 mg/kg), medetomidine (Dormilan, Axience, France, 0.028 mg/kg), midazolam (Midazolam, Aguettant, France, 0.11 mg/kg). The ultrasound unit used was Vivid i (General Electric Medical System, Tirat Carmel 39120, Israel) equipped with 3S (1.5-3.5 MHz) phased-array transducers. Valvular regurgitations were observed in all individuals, even though they were clinically healthy. They should be interpreted with much caution as regards the drugs used for anaesthesia. One individual was diagnosed with cardiomegaly and subsequently treated. Congenital cardiomyopathies are frequent in large felids and might be associated with a lack of genetic variability in captive and wild populations. However, reference echocardiographic measure ranges are lacking for snow leopards. The use of anaesthesia has to be considered in this study as alpha-two adrenoreceptor agonists like medetomidine can cause significant changes in echocardiographic measures. This study aims to share the measures performed in these individuals in order to facilitate early diagnosis in other captive snow leopards.

ORAL SQUAMOUS CELL CARCINOMA IN A CAPTIVE-BORN BEARCAT (*ARCTICTIS BINTURONG*)

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A 3-yr-old captive-born male binturong (*Arctictis binturong*) of the Naples zoo was examined due to lethargy and inappetence. The clinical examination revealed the presence of a mass 5 cm in diameter in the left jaw. Fine-needle aspirate showed groups of polygonal cells with various degrees of keratinization (squamous cells) and severe atypia compatible with a diagnosis of oral squamous cell carcinoma. CT showed extensive invasiveness of the tumour. The neoplasm was treated with electrochemotherapy, using the drug Bleomycin 10 mg/kg IV, with electro-rotation with an electro-rotator (Cliniporator, IGEA, Italy) started 9 minutes after the injection. A subsequent CT revealed the presence of metastases in the lungs and spleen.

Therefore, euthanasia was performed. Necropsy showed a widely invasive multinodular whitish mass in the left jaw associated with multinodular masses of similar appearance in regional lymph nodes, lungs, and spleen. The histopathology examination of the oral mass revealed, a multinodular proliferation of polygonal cells with various degrees of keratinization, organized in cords and nests and infiltrating normal parenchyma. Metastases were confirmed in the regional lymph nodes, in lungs, and in the spleen. Micrometastases were also found in the liver. Immunohistochemistry revealed that 100% of neoplastic cells of the primary tumour and of the metastases stained positively with an antibody against pancytokeratin (Clone AE1/AE3 cod. M3515, Dako, Santa Clara, CA, USA).

These findings confirm a diagnosis of widely invasive oral squamous cell carcinoma. This is the first report of oral squamous cell carcinoma in a binturong. Early detection of this malignant neoplasm is essential for effective surgical therapy but it can be challenging to diagnose and treat in unconventional animals.

DETECTION OF GLYPHOSATE IN EUROPEAN BROWN HARE KIDNEYS (*LEPUS EUROPAEUS*)

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The European brown hare population has been decreasing for many years. There are many factors that influence the decline of hare numbers, but the actual cause is not entirely known. The systemic herbicide glyphosate has been criticized for years to be cancerogenic and to cause health risks. It is used on agricultural land, which provides cover and food for hares, which predisposes the hares for glyphosate intake.

Due to these considerations, 63 kidneys of European hares from Austria were tested for glyphosate. The animal samples came from four different provinces in Burgenland, Lower Austria, Upper Austria and from the Research Institute of Wildlife Ecology in Vienna, where brown hares are kept for scientific purposes. During necropsies, the kidneys were examined histopathologically and samples were taken for glyphosate determination using ELISA. Samples were analyzed in duplicates and experimental errors were determined. All 63 hare samples tested positive for glyphosate. The concentration of glyphosate ranged from 0.79 to 4.67 ng/g kidney tissue, only five samples had extremely high concentrations ($n_1 = 2401.60$ ng/g and $n_2 = 3470.84$ ng/g from Burgenland, $n_3 = 28.88$ ng/g and $n_4 = 6.19$ ng/g from Lower Austria and $n_5 = 109.91$ ng/g from Upper Austria). Statistical modelling of glyphosate concentration were done in relation to provenance, sex, age, season and pathological changes of the kidneys. Glyphosate levels differed between provenances, age and season, but there was no association between glyphosate concentration and sex or pathologic findings in the kidney tissues.

An association between glyphosate and the decline of the European hare population cannot be totally excluded, so further investigations are necessary and should focus on possible influence of reproductive activity in conjunction with glyphosate intake.

RADIOLOGICAL INVESTIGATIONS OF LUMPY JAW DISEASE IN KANGAROOS FOR A TARGETED DETECTION AND CLASSIFICATION OF THE DISEASE

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Lumpy Jaw Disease (LJD) is an oral pyogranulomatous osteomyelitis that is known as one of the most common diseases in captive macropods (CONOR and KILLAGON, 2010; JACKSON, 2003; VOGELNEST and WOODS, 2008). The most common clinical presentation are mandibular or maxillary swellings with or without the presence of a draining sinus (VOGELNEST and WOODS, 2008). Predisposing factors include overcrowding, unhygienic husbandry and feeding with too soft or sharp food (JACKSON, 2003). LJD is caused by anaerobic, gram-negative bacteria e.g. *Fusobacterium necrophorum* and *Bacteroides* spp. (ANTIABONG et al., 2013).

Osteomyelitis of the mandible is a problem in human medicine, as well. The clinical presentation, radiologic appearance, and treatment of mandibular osteomyelitis vary with the stage of the disease (SCHUKNECHT and VALAVANIS, 2003).

In the literature there is no standardised protocol on how to radiograph a kangaroo's head to best detect the disease, and which diagnostic imaging modality is preferable (skull radiography, dental radiography, or computed tomography).

The aim of our research was to determine the best method to detect LJD in its early stages to initiate optimal treatment as soon as possible. It was also unclear which aspects of the disease were visible when comparing different diagnostic imaging modalities. A questionnaire was also sent to 77 zoos and animal parks holding kangaroos to collect data about occurrence, preferred examination and treatment methods of LJD.

A radiological examination with latero-oblique angles of 30 degrees by open mouth was determined as the most informative and avoided overlapping of relevant structures. Dental radiography appears the preferential diagnostic imaging modality for the detection of LJD in kangaroos.

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ENDOSCOPIC REMOVAL OF A MICROCHIP ACCIDENTALLY IMPLANTED IN THE LUNGS OF A FISCHER'S TURACO (*TAURACO FISCHERI*)

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A 9-month old, male Fischer's turaco (*Tauraco fischeri*, 230 g BW) was presented for pre-shipment examination before transfer to another zoo. Under general anaesthesia a microchip (DATAMARS, 11.4 x 2.18 mm, Datamars SA, Via industria 16, CH-6814 Lamona, Switzerland) was accidentally implanted into the left lung in an anterograde direction instead of SC in the neck region (although standard in avian medicine is IM in the pectoral muscle). Acute laryngeal hemorrhage occurred but stopped quickly. The bird was treated with 8.0 ml lactated ringers solution SC, and hospitalized for observation and supportive care. Neither respiratory nor other clinical signs occurred, but radiographs confirmed intrapulmonary position of the microchip. To train a removal procedure, a microchip was implanted the same way into the lung of a domestic pigeon's carcass and celioscopic removal of the foreign body was performed. Six days after the incident, the Fischer's turaco was re-anesthetized using isoflurane-oxygen inhalation anesthesia via facemask. A 30° oblique 2.7 mm rigid telescope with a 4.8 mm operating sheath, connected to an endovideo camera and monitor (Karl Storz, Anklin AG, Bodenmattstrasse 34, CH-4153 Reinach, Switzerland), was inserted via post-femoral access into the left abdominal and caudal thoracic air sac. The microchip was visualized in the parenchyma of the left lung lobe. Surrounding lung tissue was bluntly dissected and the microchip was pulled out using a 1.7 mm grasping forceps. Lung tissue and the implantation site were inspected for hemorrhages, but no bleeding occurred. Analgesia was provided using meloxicam (Metacam 1.5 mg/ml orale Suspension, Boehringer Ingelheim GmbH, Basel, Switzerland; 0.5 mg/kg PO SID for 5 days). Recovery was uneventful and the bird was transferred to the new zoo 14 days later.

EFFECTS OVER TIME OF FLUORESCENT LIGHTING VERSUS SUNLIGHT EXPOSURE ON CALCIUM, MAGNESIUM, VITAMIN D IN HISPANIOLAN AMAZON PARROTS (*AMAZONA VENTRALIS*)

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Low exposure to ultraviolet light, and resulting vitamin D deficiency, has recently been linked to autism spectrum disorder (ASD) in humans (BENER, et al., 2014). Captive psittacines that exhibit behavior disorders appear to share many similarities (e.g., repetitive behaviors) to ASD. The metabolic and psychological effects of housing pet parrots indoors without UVB lighting have historically not been considered in animal husbandry or veterinary care. In this study, 25-hydroxyvitamin D (calcifediol) (NAIR R, et al., 2012), ionized calcium, and ionized magnesium levels were compared between 10 Hispaniolan Amazon parrots (HAP) (*Amazona ventralis*) housed indoors with access to only fluorescent lighting and 10 HAP historically housed outdoors. While ionized calcium and magnesium levels were not remarkably different, calcifediol levels were significantly greater in the colony of birds housed outdoors. For the second investigation, calcifediol, ionized calcium, and ionized magnesium levels were reevaluated against the historically outdoor-housed HAP, subsequently deprived of natural UV light exposure, over time. The first group of birds (N=9) have been housed indoors for approximately 20 years with only exposure to fluorescent lighting. The second group of birds (N=8) have been housed under the same conditions as the first group for approximately 18 months. There were no significant differences in the vitamin D levels of the first group of parrots over time. However, the second group of parrots experienced a significant decrease in vitamin D levels over time, comparable to the level of the first group. The second group also experienced a decrease in ionized calcium.

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