Jaguar (Panthera onca) EAZA Best Practice Guidelines



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EAZA Preamble: Right from the very beginning it has been the concern of EAZA and the EEPs to encourage and promote the highest possible standards for husbandry of zoo and aquarium animals. For this reason, quite early on, EAZA developed the "Minimum Standards for the Accommodation and Care of Animals in Zoos and Aquaria". These standards lay down general principles of animal keeping, to which the members of EAZA feel themselves committed. Above and beyond this, some countries have defined regulatory minimum standards for the keeping of individual species regarding the size and furnishings of enclosures etc., which, according to the opinion of authors, should definitely be fulfilled before allowing such animals to be kept within the area of the jurisdiction of those countries. These minimum standards are intended to determine the borderline of acceptable animal welfare. It is not permitted to fall short of these standards. How difficult it is to determine the standards, however, can be seen in the fact that minimum standards vary from country to country. Above and beyond this, specialists of the EEPs and TAGs have undertaken the considerable task of laying down guidelines for keeping individual animal species. Whilst some aspects of husbandry reported in the guidelines will define minimum standards, in general, these guidelines are not to be understood as minimum requirements; they represent best practice. As such the EAZA Best Practice Guidelines for keeping animals intend rather to describe the desirable design of enclosures and prerequisites for animal keeping that are, according to the present state of knowledge, considered as being optimal for each species. They intend above all to indicate how enclosures should be designed and what conditions should be fulfilled for the optimal care of individual species.

Picture front page: Jaguar at Chester Zoo

Introduction

Much information for these EAZA Best Practice Guidelines is taken from the comprehensive AZA Jaguar (*Panthera onca*) Care Manual created by the AZA Jaguar Species Survival Plan (2014) in association with the AZA Felid Taxon Advisory Group - many thanks to all who contributed to that document.

Additional edits, suggested content and photo contributions were made by the following:

- Douglas Richardson, Head of Living Collections, RZSS, Highland Wildlife Park,
- John Lewis, International Zoo Veterinary Group,
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- Dave Hall, Team Manager, Chester Zoo,
- Cathrine Sauer, Nutritionist, Chester Zoo,
- Alex Sliwa, EAZA Felid TAG Chair.

The veterinary section was written by our Jaguar EEP Veterinary Advisor, Melissa Nollet from Bellewaerde Zoo in Belgium. Many thanks to Melissa and all the EEP participants who completed our Jaguar veterinary survey in 2019 to help inform these guidelines. Additional thanks go to María José Duque-Correa for conducting a retrospective review of postmortems from jaguars housed in EAZA collections between 1998 and 2018, which also provided useful information for this document.

For any edits, additions or comments about these Best Practice Guidelines, please contact the editor, Rebecca Biddle.

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Section 1: Biology and Field Data

<u>Biology</u>

1.1 Taxonomy

Table 1: Taxonomy of Panthera onca.

Classification	Taxonomy
Kingdom:	Animalia
Phylum:	Chordata
Class:	Mammalia
Order:	Carnivora
Suborder:	Feliformia
Family:	Felidae
	Pantherinae
Common Names:	English - Jaguar
	Spanish - Tigre Real, Tigre Americano, Otorongo, Yaguar, Yaguarete

The jaguar (*Panthera onca*) is the only living representative of the genus *Panthera* found in the New World (Nowell and Jackson, 1996). This genus also includes tiger (*P.tigris*), lion (*P.leo*), leopard (*P. pardus*), and snow leopard (*P. uncia*).

Although eight subspecies have been recognised (Seymour, 1989), genetic (Eizirik *et al*, 2001) and morphological (Larson, 1997) analysis finds no support for the existence of discrete subspecies. Whilst not sub species level, evidences suggests the presence of four incompletely isolated phylogeographic groups: Mexico and Guatemala, southern Central America, northern south America, and South America south of the Amazon river (Eizirik *et al*, 2001, Quigley *et al*, 2017).

For the purposes of captive management and public education the EEP considers *Panthera onca* a single species without subspecies designation.

1.2 Morphology

1.2.1 Body Size

The jaguar is the largest cat in the Americas and the third largest in the world. It has a record weight of over 158kg (Emmons, 1997). Its head and body length (without tail) can be up to 1.85m and the tail can measure 75cm. The height at the shoulder may be up to 75cm (Nowak & Paradiso, 1983).

1.2.2 General Description

The jaguar's coat colour ranges from pale yellow to reddish brown, with a much paler (often white) underbelly. It has spots on the neck, body and limbs that form rosettes, often containing black markings within them. On the head and underparts, the spots are simple black dots.

Black or melanistic jaguars are not uncommon and are recognised as a colour morph of the same species. Their difference in colour is caused by a dominant allele (rather than a recessive allele as in leopards). The typical rosette markings are still present on black jaguars, just hidden by an excess of the black pigment melanin, but often visible in bright light.

Compared to a leopard, the jaguar is stocky and more powerfully built. It has a square jaw and prominent cheeks, along with robust, muscular limbs.



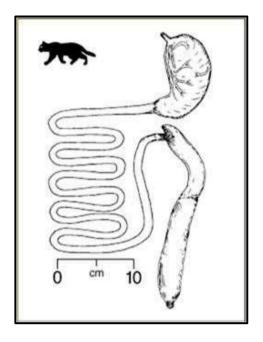
Two jaguars at Chester Zoo.

Two jaguars at Salzburg Zoo.

1.3 Physiology

1.3.1 Digestive System

Jaguars are obligate carnivores, possessing a simple digestive tract comparable to the digestive system of other carnivores including the domestic cat (Figure 3) and the lion. Among these three species, the ratio of small to large intestines is similar, with the contribution of the length of each area (stomach, small intestine, large intestine, cecum, and colon) to the total length of the tract being the same (Seymour, 1989; Smith *et al*, 2006).



Digestive tract of *Felis domesticus* (Stevens & Hume, 1995).

The intestine of domestic cats maintains bacterial colonies comparable to those in herbivorous species (Brosey *et al.*, 2000). These colonies provide protection against bacteria, they stimulate gastrointestinal function such as immunity and motility, and they digest fibre sources to produce volatile fatty acids (Suchodolski, 2011). It is likely that this is true for jaguars as well.

The felid digestive tract allows for storage of large meals in the stomach and efficient digestion of vertebrate prey (Bennett *et al*, 2010; Clauss *et al*, 2010;Smith *et al*, 2006; Vester *et al*, 2010) but may limit digestion of more complex fibre sources which omnivores and herbivores are able to utilise (Edwards *et al*, 2001; Wynne, 1989).

More recently, the fibre type qualities of indigestible prey parts including raw bones, tendons, cartilage, skin, hair or feathers, or "animal fibre" acting as either

soluble or insoluble fibre, have been suggested to be beneficial to gastrointestinal health of cheetahs and would be expected to benefit jaguars as well (Depauw, 2013).

1.3.2 Reproductive Physiology

Zoos have held jaguars for many years, but still, relatively little is known about their reproductive characteristics. Generally, females reach sexual maturity at one to two and a half years of age (Tewes and Schmidly, 1987). Males reach sexual maturity at three to four years of age (Ewer, 1973). A typical oestrus length is six to seventeen days with an oestrus cycle of 37 days (Wildt, Brown and Swanson, 1998). Oestrus can be detected by a number of behavioural cues, such as lordosis, flehmen, vocalisation, rolling, and increased scent marking. Hormone levels can be detected non-invasively by the collection and analysis of faecal and urine samples for oestrogen and progesterone metabolite concentrations (Brown *et al*, 2001).

1.4 Longevity

Jaguars can live to between 12 – 15 years in the wild. Maximum longevity in captivity for jaguars is 28 years (Weigl, 2005).

Field Data

1.5 Zoogeography & Ecology

Much of the below information is taken from the IUCN Red List where the species is listed as Near Threatened with a decreasing population size (Quigley *et al*, 2017).

1.5.1 Distribution



Jaguar range map - historic vs current (Panthera, 2016) Historically the jaguar is believed to have ranged from the south western US through the Amazon basin to the Rio Negro in Argentina.There are still some vagrants close to the Mexican border. Its extent of occurrence is estimated at 8.75 million km², with its stronghold being the rainforest of the Amazon basin. This stronghold comprises 88% of all remaining jaguars (Quigley *et al*, 2017). However, ecological models have indicated that much of the Amazon basin is of low habitat suitability, compared with the Pantanal, Paraguayan Chaco, and Caatinga (Torres *et al.* 2007).

The species has been virtually eliminated from much of the drier northern parts of its range, as well as northern Brazil, Argentina's pampas scrub grasslands and Uruguay, and it is now estimated to occupy only about 46% of its historic range (IUCN 2017, Sanderson *et al.* 2002).

Areas important for conservation of viablejaguar populations have been defined by Sanderson *et al.* (2002) - Jaguar Conservation Units or JCUs, which consist of 51 areas add up to 1.29 million km², or 13% of jaguar range.

1.5.2 Habitat

The home range of wild jaguar has been established to be 25–38 km² for females and at least double for males (Schaller and Crawshaw, 1980). The species is associated with the presence of water and its habitats range from rainforest to seasonally flooded swamp areas, pampas grassland, thorn scrub woodland, and dry deciduous forest (Nowell and Jackson 1996). In Belize, jaguars are reportedly more abundant in lowland areas of relatively dense forest cover with permanent water sources than in open, seasonally dry forests.

Although jaguars have been reported in elevations as high as 3,000m (Brown and Lopez Gonzales 2001), they generally avoid montane forest, and haven't been found in the high plateau of central Mexico or above 2,700m in the Andes. In studies from Brazil, Peru, Colombia and Mexico, summarised by Sunquist and Sunquist (2002), density estimates ranged from 1.7 - 4 adults per 100km². Density estimates by Silver *et al.* (2004) from five different study sites, ranged from 2.4-8.8 adults per 100km², with the highest density found in Belize's Cockscomb Basin Wildlife Reserve (rainforest), a density similar to the 6-8 per 100km² found by Rabinowitz and Nottingham (1986). The latter study found female home ranges of 10km², were overlapped by male home ranges which varied from 28 to 40km².

1.5.3 Population and Conservation Status

The IUCN classifies the jaguar as Near Threatened, due to a suspected decline of 20-25% in the area of occupancy, over the past three generations (Quigley *et al*, 2017). The species has been under considerable pressure because of conflict with the livestock industry in Latin America for many years and its population is in decline. Additionally, deforestation rates are extremely high in Latin America and habitat fragmentation isolates jaguar populations making them more vulnerable to human persecution (Nowell and Jackson, 1996). In order tackle the major threat of habitat fragmentation, an ambitious habitat corridor programme has been launched to help conserve a continuous north to south habitat corridor through the species range (Rabinowitz, 2007).

It has been reported that jaguars are frequently shot on sight, despite protective legislation (Nowell and Jackson, 1996). A large proportion of jaguar range has a depleted prey base and thus jaguars are known to kill cattle – in response jaguars are sometimes killed by ranchers as a pest species. The vulnerability to persecution is demonstrated by the jaguars' disappearance by the mid-1900's from the south-western US. Addressing livestock management is a high priority conservation actions in many range countries.

Commercial hunting and trapping of jaguars for their pelts has declined drastically since the mid-1970's, when anti-fur campaigns and CITES controls progressively shut down international markets (Nowell and Jackson, 1996). Although hunting has decreased there is still demand for jaguar paws, teeth and other products.

The jaguar is included in CITES Appendix I, and is fully protected at the national level across most of its range. Hunting is prohibited in Argentina, Brazil, Colombia, French Guiana, Honduras, Nicaragua,

Panama, Paraguay, Suriname, United States, and Venezuela. Hunting restrictions are in place in Brazil, Costa Rica, Guatemala, Mexico and Peru (Nowell and Jackson, 1996).

1.6 Diet and Feeding Behaviour

1.6.1 Food Preference

Jaguars utilise all areas, day or night, including forests, river and lake edges (Emmons, 1987). Their activity patterns coincide with the behaviour of their main prey species (Harmsen *et al*, 2010). Activity can range from 30 - 60% during daylight hours. In general, jaguars are considered opportunistic predators, and they will often walk extensively until prey is encountered (Emmons, 1987). Similar to most *Felidae* they obtain prey by stalking or ambushing. Their diet is diverse and depends on geographic location. Prey size can range from small (less than 1-2 kg) to large (greater than 10-15 kg) though most are greater than 1-2 kg (Emmons, 1987; Rabinowitz and Nottingham, 1986).

Prey species in the wild commonly include: capybara (*Hydrochoerus hydrochaeris*), caiman (*Caiman crocodilus* and *C. yacare*), side-necked turtles (*Podocnemis vogli* and *Podocnemis unifilis*), collared peccary (*Tayassu tajacu*), white lipped peccary (*Tayassu pecari*), armadillos (*Dasypus novemcinctus*), paca (*Agouti paca*), and coati (*Nasua nasua*).

1.6.2 Feeding

When feeding, a jaguar may stay with its prey for one to three days, may move the prey, or abandon it shortly after obtaining. Depending on prey size, it may take several days between feeds. Schaller and Crawshaw (1980) document six kills in a period of 35 days for one individual. Many prey parts are entirely consumed. Feet, hooves and bone pieces up to 4.4cm, as well as armadillo and anteater claws (*Tamandua mexicana*) can appear in the faeces. Exceptions include skulls along with larger bones, digestive tracts and carapace of turtles and armadillos (Emmons, 1987; Rabinowitz and Nottingham, 1986). Field biologists commonly estimate that use 34g of prey is required per 1kg of jaguar body weight to assess suitability of habitat (Novack, 2003, Polisar *et al*, 2003).

1.7 Reproduction

1.7.1 Sexual Maturity

Females reach sexual maturity at 1 - 2.5 years of age (Tewes and Schmidly, 1987). Males reach sexual maturity at 3 - 4 years of age (Ewer, 1973).

1.7.2 Seasonality of Cycling

Jaguars have been reported to give birth between March and June in the Southern Pantanal (Quigley and Crawshaw, 2001), indicating that mating occurs between December and March, which coincides with receding of the floodwaters, and greater availability of prey. The reproductive hormones of males and females appear to increase during the receding of the floodwaters (Morato *et al*, 2004).

1.7.3 Gestation Period

Gestation varies from 98 to 111 days (Ewer, 1973). Generally, one or two cubs are born.

1.8 Behaviour

1.8.1 Activity

The jaguar is primarily nocturnal in its feeding and movement (Schaller and Vasconcelos, 1978; Mondolfi and Hoogesteijn, 1982). Jaguars tend to rest between mid-morning and afternoon, but some daytime activity and movement is fairly common, and they will even hunt during the day if needed.

1.8.2 Predation

The jaguar may utilise a characteristic *Panthera* killing technique; attacking with a deep bite to the throat to suffocate prey. But more often than not, an attack where a bite pierces the back of the skull at its weakest point is used. The jaguar will then often drag the prey to a thicket or secluded spot, removing the digestive tract 2 - 3 m away. The ventral surface of the prey is eaten first: neck, chest, heart and lungs, and then the shoulders (Schaller and Vasconcelos, 1978).

For reptiles, a slightly different technique is recorded, with the jaguar pouncing on the prey from behind, immediately biting through the neck and severing the cervical vertebrate, rendering the reptile unable to lash itself into the water. If eating a turtle, the jaguar will force its paw into the shellthrough the opening between the carapace and plastron and scoops out the flesh without breaking

the shell. In all cases the jaguar attacks from cover, and usually from a blind side with a characteristic pounce.

1.8.3 Social Behaviour

Jaguars are solitary animals, holding individual home ranges. Male jaguars in the Pantanal Region of Brazil were reported to have home ranges of twice average seen elsewhere within their range, (28 to 40km) and females moved over a minimum area of 10km. Typically, adult males had a home range encompassing two or three females' territories, defending it against all jaguars except sub- adults and females (Schaller and Crawshaw, 1980). If a jaguar dies, a jaguar that had a contiguous home range may fill that vacant home range, and the vacant home range may then be filled in turn by a jaguar from an outside territory (Rabinowitz, 1986).

1.8.4 Sexual Behaviour

When a female enters oestrus she may venture outside the normal home range and as a result be courted by several males (Nowak, 1991). There are reports of a 13-year-old wild female being found with a cub (Brown and Lopez-Gonzalez, 2001).

Section 2: Zoo Management

Zoo Management

2.1 Enclosure

Prior to committing to and designing a new jaguar exhibit, institutions should consult the EAZA Felid TAG to identify which species have the greatest need for the additional spaces they will be providing. This will ensure that the collection is considering the TAG's programme priorities.

Careful consideration should be given to exhibit design so that all areas meet the physical, social, behavioural, and psychological needs of the species. Animals should be presented in a manner reflecting modern zoological practices in exhibit design and all animals must be housed in enclosures and in appropriate groupings that meet their physical, psychological, and social needs. Furthermore, it is beneficial to design enclosures in a way that allows flexibility, and addition or removal of structures, which can be changed in response to the individual's behaviour and enclosure use.

Jaguars can readily acclimatise to a range of environmental conditions. However, if given time to do so, they must be protected from any adverse environmental conditions as problems can arise when suddenly presented with a large change in conditions.

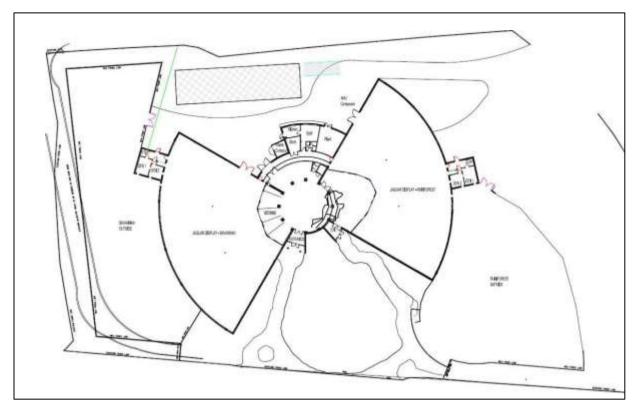
It is strongly recommended to provide indoor and outdoor access for jaguars and that pairs of enclosures are built to allow extra holding areas if needed. This allows flexibility in management and for extra space if occasionally required, for example to separate a mother with cubs from the sire, or incompatible animals from one another.

2.1.1 Dimensions

Throughout this section, examples are given using the two modern jaguars' facilities - one at Chester Zoo (The North of England Zoological Society) and one that is newly constructed at Paradise Wildlife Park (The Zoological Society of Herefordshire). These examples give an idea of the space and facilities needed to house jaguars. It is recommended that an outdoor area for jaguars be of >200m² plus an extra 50% for each extra specimen. Minimum requirements for den size are 5.8m² surface area (2.4m x 2.4m), height 2.4m.

An inside enclosure of >200m², height of 4m, should be provided plus an extra 50% for each extra specimen and two outdoor enclosures in order to manage individuals separately if necessary.

At Chester Zoo the outdoor areas are 1200m² each. Additionally, the indoor enclosures have areas of 820m² and 760m². The dens have areas of 12m² and 9m². The outdoor enclosures are confined with a 5m high wire-mesh fence (see details below).



Drawing of jaguar facilities at Chester Zoo.



Arial photograph of jaguar facilities at Chester Zoo.

The new "Jaguar Jungle" exhibit at Paradise Wildlife Park is a fully enclosed enclosure and split into 5 different areas. These areas are as follows:

- The main outside area which is roughly 2/3 of the overall outside space
- A separation pen area roughly 1/3 of the overall outside space
- One on show indoor den area
- Two off show indoor den areas

This exhibit has been designed in such a way that nearly all the inside exhibit space can be utilised by the cats including its height, but the cats have limited access to the boundary fence itself except at ground level. This makes it as difficult as possible for cats to test the boundary fence line on the ground, without limiting the space the jaguars can use. The overall dimensions of the exhibit are as follows:

- Outdoor: 30m x 30m = 900m² of floor space, 6m height with a fully enclosed roof allowing for multiple levels throughout the exhibit.
- On-show indoor den area: 5m x 5m = 25m² of floor space, 6m height and fully enclosed allowing for multiple levels.
- Off-show den area: 3m x 2.5m = 7.5m² of floor space, 4.5m height with 2 platform levels in each den.



Design of the new "Jaguar Jungle" exhibit at Paradise Wildlife Park



Impression of the new "Jaguar Jungle" exhibit at Paradise Wildlife Park



Impression of the new "Jaguar Jungle" exhibit at Paradise Wildlife Park

2.1.2 Enclosure Characteristics

Zoos located in colder climates, where the temperature drops below 10°C, are required to have indoor exhibits in addition to outdoor exhibits.



SHELTER: Providing sections of varying shelter and sunlight exposure in an exhibit can create temperature zones allowing the animals to selectthe most comfortable location – this can be accomplished by using a rock or other object that offers protection from prevailing wind, while offering a sunny exposure. When kept indoors, jaguars should be protected from temperatures exceeding 30°C through the use of circulating fans orair conditioning. Clinical signs associated with poor ability to handle low temperatures may include huddling/shivering, increased pacing, and/or constipation. Multiple shelters should be offered if more than one animal is sharing an enclosure, and shade should always be available when animals are outside.

HEATING: Heating systems should be carefully considered due to the destructive nature of jaguars, i.e. heat lamps and other portable heat sources can be destroyed if the animals gain access to them. Forward planning allows the use of flexible heating hose embedded in concrete, for example adding a warm rock constructed of concrete, where the tubing is adequately protected from the animals. However, given that animals are acclimated and have adequate shelter from wind, jaguars can tolerate conditions down to -1° C.

DENS: Where possible, collections should have more dens available than the number of jaguars they keep, for example if keeping one animal, two dens should be available, if three animals, four dens should be available. At the very least, every animal should have its own individual den containing a shelf or box, which can be used if the animals need to be kept out of their main enclosure for maintenance, for example. Every individual should be able to seek and find its comfort zone 24/7. A means of remote observation within the den should be incorporated, which can be a simple small viewing port, or closed-circuit video monitors. Logs and stumps for scentmarking and sharpening claws are also recommended for night holding enclosures. The minimum recommended size for shift cages used in daily operations should measure no less than $2.45m \times 2.45m \times 2.45m^2 = 6m$. Dens should be designed to prevent accidental contact that would allow the tail or a limb to enter the cage of an adjacent, incompatible cat, resulting in injury. If they are directlynext to each other, a solid divider is the best option. If mesh is used, it must be no less than 4mm (6- gauge) composition with a grid measuring no more than 1.5cm x 1.5cm. Doors should be designed to prevent tail injury during transfer procedures, the easiest way to prevent this is to be sure there is a clear unobstructed view of the shift door. Optional openings where animals can see and smell but not touch each other should be considered between individual holding units to facilitate

introductions. At Paradise Wildlife Park the dens are designed so that they can be used for breeding or for the introduction of new cats in a more controlled way if needed. All have platforms at varying levels to give the cats as much choice as possible. There is a keeper corridor that runs in front of the dens so keepers can check the dens are clear before servicing the area and access to the house is with a double gate airlock system to ensure the corridor is secure and clear before entering the house itself.

WATER: Water features are recommended and should be accessible year-round to provide an opportunity for jaguars to thermoregulate - if misters or sprinklers are used, there must always be a place for the animal to retreat. The clinical signs of heat stress are: increased respiration, panting, lethargy, anorexia, diarrhoea, and / or constipation. Where possible, water systems should be clean and capable of being a drinking water source. Collections must have a regular programme of monitoring water quality, and keep a written record of long-term water quality / any chemical additions. It is not uncommon for jaguars to defecate in the water, so a second, easily flushable drinking source should always be available. For example, the facilities at Paradise Wildlife Park include a 5m x 3m pool which is 1m deep at its deepest point. The pool is positioned next to a window to allow the public to watch the jaguars under water should they choose to use it. The pool has mechanical filtration in the form of a sand filter and a chlorine dosing unit should it be needed in the summer months. The chlorine unit doses at a lower rate than tap water and regular testing and record keeping will be used to ensure chlorine levels stay at a safe level.

LIGHTING: Careful consideration should be given to the intensity, and duration of light. Outdoor enclosures are ideal as they allow natural lighting. Indoor lighting, should be protected with mesh barriers, as should skylights, as primary containment. For safety considerations, light levels should be appropriate to provide unrestricted viewing of the animals by the staff at all times, especially during night house operations. Most large felids adapt well to normal light cycles, and usually do not present negative behaviours. At Paradise Wildlife Park there is lighting in the den areas for keeper use when the dens are serviced, and emergency floodlights thatcover the outside areas for emergency use only in the winter months or at night time. The den lights are either situated outside of the dens or have been secured flush in the walls/celling to prevent the cats from accessing them.

VENTILATION: Proper ventilation is an integral part of exhibit design. It can help thermoregulation, control odours, and can potentially reduce the risk of disease transmission among specimens. Cat urine contains a small amount of ammonia and anhydrous ammonia gas forms vapours that are heavier than air - to prevent a build-up of this potentially harmful gas at floor level in dens, attention must be paid to the effectiveness of airflow. For example, at Paradise Wildlife Park, there are extractor fans situated inall the dens allowing for ventilation throughout the house area. These have been built into walls with mesh covers and have been placed in areas not accessible for the cats.

SOUND: It is possible that sounds may have an effect on jaguars in zoos, for example loud noises and unusual activities such as nearby construction, machinery or large vehicles, may act as stressors. Therefore, considerations should be given to control all sounds/vibrations that can be heard by the jaguars. Even voices and activity of unfamiliar personnel in off-exhibit or night house areas may visibly disturb jaguars, which may be seen in inappetence, aggression or refusal to shift. Observations of self-trauma, stereotypic pacing and/or vacant staring also are often indicators of environmental stress. Good communication between animal care staff and other zoo divisions can ensure that any unusual or loud noises are timed or monitored by appropriate staff, and their effectslessoned.

2.1.3 Boundary

Jaguars should not be kept in mixed species or free-range exhibits and exhibit design must be considered carefully to ensure that all areas are secure, with particular attention paid to doors, gates, keeper access doors, locking mechanisms, and barrier dimensions and construction.

Facilities for jaguars should be designed to provide the highest level of security. Dry moats should have a width of no less than 8m and vertical jump walls at least 4.5m high are recommended. Cantilevered supports with mesh or fencing material with an attached hot-wire is recommended for open-top fenced exhibits, or an impenetrable roof, no lower than 3.5m. Jaguars are great climbers and jumpers, thus great care should be taken in placement of landscaping and exhibit furniture to avoid the possibility of their use by cats to reach areas in which the public, other animals or staff may be injured. Enclosure design must also prevent animals jumping from one landscaping element to another, in order to reach a location otherwise out of reach. Jaguars are not known to dig routinely, however, for extra care a buried 1m chain-link on the interior could be considered.

Secondary containment in keeper areas is recommended and can consist of a safety door constructed of a mesh type described previously. A good rule of thumb to follow is that an animal should always have at least two doors between its enclosure and potential escape. Likewise, a clear field of view should be preserved at all times for keepers to observe animals' locations. If a safety door is not feasible, a way to look into the holding area, such as through safety glass, allows keepers to be certain everything is in order prior to entering. All parts of the holding area behind the safety glass must be visible.

Fence or mesh material should be no less than 4mm (6-gauge) steel wire composition, with good results achieved using 5cm x 10cm mesh. Custom woven stainless steel cable net is also available with a cable diameter of 2.3mm or more, in a 5cm or 7.5cm mesh. Lighter-weight mesh is not appropriate for this species. Because of the risk of jaguars' paws reaching through, a mesh measuring no more than 5cm x 5cm is recommended in keeper work areas or zones in which the public may be able to reach it. In keeper work areas, good results have been achieved using woven steel wire grids of 5cm x 5cm composed of 6.3mm wire stock. It should be noted that a woven cable net is quite flexible and jaguars can stretch it as much as several feet outward when striking the barrier at a run.

Exhibits in which the visiting public are not intended to have contact with animals must have a guardrail / barrier that separates the two. Secondary guardrails should be used wherever the potential exists for public contact with primary containment fencing or mesh materials, or moated exhibits. They should be placed 1.5m from the cage front. Public viewing points composed oftempered glass are commonly used and do not require the use of secondary guardrails. If glass is used it is recommended that this be at least 33mm thick and laminated. In order to protect glass from scratches caused by the animals, a very thin electric wire on the inside of the glass has shown to prevent the animals from lying down directly next to the glass.

Below are the fence specifications at Chester Zoo. Both outdoor jaguar enclosures are around1200m² confined with a 5m high wire-mesh fence with deflectors at the edge, pointing in a 90° anglefrom the fence into the exhibit. The fence is protected with hot-wire to prevent the Jaguars from climbing on it. The fence is steel and thick chain link. All measurements are in millimetres. Abbreviations as follows - SHS: Square hollow section, RHS: Rectangular hollow section, RSA: Rolled Steel Angles.

- Main Fence: 5000 above ground (100 x 60 x 5 RHS), 250 underground (100 x 60 x 5 RHS) screwed into concrete base
- Concrete base: isolated pad base (1500 x 1000 x 1000)
- Overhang: depth 1600 (100 x 60 x 5 RHS welded to fence post with 6 mm constant filet weld all round)
- Hot wires: 14 hot wires, 6 at 500 above ground then at 500 intervals, 8 at 250 intervals
- Supports: 3600 (60 x 60 x 5 SHS) (plus 250 underground in concrete base) situated at 3000 intervals along the fence
- Chain link: 50 width, 5 gage



Main boundary fence of jaguar enclosure at Chester Zoo, UK.

2.1.4 Substrate



Young jaguar at Bratislava Zoo.

To maximize available square footage of naturalistic exhibits, natural or artificial complex features entering the vertical plane, e.g., connected tree branches, should be used.

Exhibits should be designed to reflect the naturally occurring felid behaviours i.e., hunting, resting, territoriality, scent marking, and the defense of home range against conspecifics.

Jaguars are comfortable in trees as well as on the ground, enclosure designs should therefore incorporate climbing structures of live, dead and artificial trees. Artificial snags or ledges should be incorporated into the exhibit design to produce elevated resting sites, as well as long distance viewing. At least one resting site per animal should be provided and each should be large enough to comfortably allow the cat to stretch out and be stable.



Climbing structures at Paradise Wildlife Park and jaguars at Paris Zoo.

Artificial and natural rocks can be used to provide visual and auditory barriers, which when used in conjunction with other features should produce multi-level, complex pathways that can help to prevent the development of stereotypic behaviours. Exhibits should be designed to minimise psychological pressure from viewer; furnishings such as vegetation, rockwork, and climbing structures may help reduce stress. Landscaping should be maximised to simulate natural cover and provide walkways, escape routes, and shade.

Plant toxicity should be ascertained prior to planting an enclosure with landscaping materials. Water features are highly recommended, and should contain deep (>1 m) and shallow areas to stimulate play activity.

Natural substrates such as grass and dirt are recommended for outdoor exhibits, but substrates such as dirt, sand, wood chips, and mulch have also been used successfully. The use of bark chips and similar mulch is highly recommended as being better for pad health, for indoor exhibits, but full replacement 1-2 times per year may be needed, depending on the size of the area.

For example at Paradise Wildlife Park, there are multiple different substrates throughout the exhibit, including, the on show den is deep litter bark chip in the on show den, and a mixture of grass bark chip and gravel outside. A lot of attention has been paid to allowing jaguars to access multiple levels, both inside and outside, through landscaping to create soil/earth mounds and has a mixture of large, real rocks and boulders as well as some fake themed rock work to try and make the exhibit look as natural looking as possible.



Substrates used at Paradise Wildlife Park.

2.1.5 Furnishings

The same careful consideration regarding exhibit size and complexity, and its relationship to the jaguars' overall well-being, must be given to the design and size of all enclosures, including those used in exhibits, holding areas, hospital, and quarantine. All exhibits should have doorways large enough to permit the placement and removal of landscaping and furniture items (e.g., rocks, deadfall, substrates, trees, etc.) Studies of wild jaguars reveal peaks in activity around 03:30–06:00 hours and 18:30–21:00 hours (Law, 2009), thus all exhibits should be designed to incorporate these nocturnal and crepuscular activity peaks. The following exhibit components can be included within jaguar enclosures to promote species-appropriate behaviours:

OUTDOOR: Rocks, bamboo, trees, swinging or hanging logs, shrubs, deadfall trees, tree limbs at different levels, rock den, concrete cave, timber platform, straw bed, rock ledge, brush pile, hammock, hot rock, gunite rock/trees, tree stumps, vines, plants, pool.

INDOOR: Wooden bench, shelves (wooden, gunite, plywood, metal) pallets, kennel, den, vines, hide box, ropes, hanging cedar logs, hanging tire, wooden perching, pool (approximately one meter deep).

CLIMBING STRUCTURES: Jaguars, like all cats, exhibit periods of activity (e.g., hunting) followed by usually longer periods of inactivity, and their spaces should be designed to accommodate this. Enclosures should incorporate climbing andhave plenty of vertical perching on branches or rocks at variousheights. Real trees and logs are essential to claw maintenance and they are more comfortable to rest upon and climb than artificial structures. High resting areas allow a cat to get above the head level of the visitor, a less psychologically vulnerable position and height that has lateral pathways, such as tree branches, becomes more useful by increasing the total available square footage.



Jaguar at Chester Zoo.

There should be plenty of visual barriers for the animal to hide from the public, and it is important that the public does not have access to all sides of an outside enclosure. Behavioural changes should be noted, and efforts should be made to minimise all conditions causing the animals to display signs of stress – indicators could include loss of appetite, aggression, self-trauma or refusalto shift. The primary keeper will usually have the best awareness of any deviation from the normal behaviour of each specific animal under his or her care.



Jaguar at Chester Zoo.

WATER SOURCES: In the wild, the jaguar is very dependent on water, and consequently has a preference for the immediate vicinity of watercourses and lagoons. Jaguars will often seek relief from the heat in rivers, and have been observed swimming across rivers while carrying a prey item. Under zoo management, there should be at least one water source. Pools, ponds, waterfalls, and streams are recommended for drinking, playing, and temperature regulation.



Water features are highly recommended, and should contain deep (>1 m) and shallow areas to stimulate play activity. Fitting an aerator to outside ponds will prevent the pond from freezing over, and prevent excess build-up of pond weed. This will also prevent ice formation creating a possible escape route.

Waterfall and pond in jaguar enclosure, Chester Zoo.

TRANSFER DOORS / CHUTES: Transfer doors and chutes are necessary to safely and effectively manage large carnivores, they should be as simple and secure as possible, and designed to prevent accidental contact between jaguars and keepers. Additionally, they should be designed to prevent tails or limbs being injured by their closing. Doors may move horizontally (sliders) or vertically (guillotines) and design is often driven by available space. Each type has advantages and drawbacks, and both styles can be obstructed by debris. Heavy guillotines are difficult for staff to operate and pose a risk of animal injury if they fall or are dropped.



Examples of open and closed horizontal slides in Amur leopard enclosure at Bellewaerde zoo.



Examples of open and closed guillotine slides in Amur leopard enclosure at Bellewaerde zoo.

Doors operated by cable and pulley systems should be inspected regularly for wear, as a door with a broken cable may move freely. Pins that go through both door and frame are recommended to secure the door in place (open or closed) increasing safety and security. Pins can be operated manually or designed with a spring mechanism to insert the pin automatically when pin and hole align, however this usually causes a bang when the pin springs back into places which can be annoying to the jaguar.

Doors may be electric, hydraulic or manually operated. Well-designed manual doors are the most costeffective choice and do not require as much specialised maintenance. Electric doors should have a manual or battery backup for use during power outages. Regardless of design, door controls must be in a location allowing the keeper to have good visibility of the door and of animals passing through it. Animal doors should be no smaller than 50cm x 90cm.

At Paradise Wildlife Park all of the hatches are slider hatches (as opposed to guillotine) that run on a hanging ball bearing rail. These hatches use guides only on the floor level meaning they do not get debris, dirt, etc. in floor runners as they are not needed. The hatches secure into a steel plate when closed which the cats have no access to them meaning they are extremely secure when in the closed position as well as easy to maintain. All hatches are a double hatch system, one is a mesh door and one is a solid door which allows for slower introduction of cats during mixing, blocking of cats from viewing through hatches during vet visits and adds extra safety to keepers when working inside the cat areas as both hatches are secured independently.

2.1.6 Maintenance

Animal care staff should never enter an enclosure with adult or sub-adult jaguars. For the purposes of cleaning and making changes to the environment, for example adding enrichment, each facility should have a means to move the animal from the exhibit to an equally secure off-exhibit space, such as a holding area.

Outdoor enclosures and indoor enclosures should be inspected daily to remove any faecal material and waste products, and a thorough cleaning of indoor areas should occur at least once a week.

2.2 Feeding

Diet formulation criteria should address the animal's nutritional needs, feeding ecology, as well as individual and natural histories to ensure that species-specific feeding patterns and behaviours are stimulated. Food should be purchased from reliable, sustainable and well-managed sources. The nutritional analysis of the food should be regularly tested and recorded. Chester Zoo's jaguar Diet Sheets are given as examples in Appendix 1.

2.2.1 Basic Diet

It is suggested in the AZA Care Manual that 1.8kg prey for male and 1.6kg prey for female adult jaguars should be given per day. The availability of energy in a feed will depend on how digestible the food is, for example if feeding carcasses, skin, bones, feathers etc. all "bind" some of the energy which means

that animals need to eat more whole prey to get the same amount of energy as in a meat type product. Thus, individual feeding rates should be evaluated and readjusted based on regular assessment of body condition score and weights. Fasting for 24 hours without access to food is part of the feeding management at some institutions.

Food items from non-domestic and domestic stock should be frozen prior to feeding to kill any parasites that might be present. Meat-based diets should not be allowed to warm to room temperatures or above for extended periods of time, as this may result in the growth of harmful bacterial organisms. The best way to thaw frozen meat is at $4^{\circ}C - i.e.$ in a fridge.

Some collections use nutrient supplements, for example Paradise Wildlife Park use Canivit7 supplement, dosed at the bodyweight of the cat (see below picture) and Chester Zoo use Mazuri Carnivore Supplement.



Canivit7 supplement, used by Paradise Wildlife Park, dosed at the bodyweight of the cat.

2.2.2 Method of Feeding

The diet of wild jaguars is whole prey, thus providing humanely-killed animals to jaguars can promote a wide range of species-appropriate hunting, food manipulation, and feeding behaviours. Whole animals, gutted carcasses, or carcass fragments should be provided.

Many felids will perform all or part of their species-appropriate 'stalk-rush-kill' behavioural sequence when presented with carcasses (Law, 2009). Carcass feeding may promote physical health (e.g., improved dental hygiene) as well as psychological well-being.

Collections may choose to remove guts from prey that have been frozen, as there is a risk that if the freezing and thawing process has not been done very carefully, there is a risk that the guts could

harbour bacteria.

food source)

The hide/fur, cartilage, gut and gut contents are more similar to the natural diet of jaguars than hard bones and may therefore be more appropriate. Additionally, the fibre-type qualities of indigestible prey parts including raw bones, tendons, cartilage, skin, hair or feathers, or "animal fibre" act as either soluble or insoluble fibre (Depauw *et al.*, 2012). Fermentation of these items in the colon has been suggested to be of benefit to the gastrointestinal health of cheetahs and would be expected to benefit jaguars as well (Depauw, 2013).

Enriching foods consumed by jaguars should be considered a part of the diet. All dietary enrichment should go through an institutional approval process, including review by nutritionists and veterinarians. All new items should be monitored closely when first provided. Ice should be usedwith caution considering several cases of tooth damage in domestic and exotic carnivores treated by zoo dentists (Briggs and Scheels, 2005). See below table for a list of examples of food items used and avoided by European collections:

Food items used by European collections Food items not used by European collections Chicken (whole / feathered) Food items with unknown sources • Rabbit (riffle shot) • • Lead shot game e.g., duck, pheasant Hare (riffle shot) Pork neck / hock • • Horse meat (on bone) • • Calf • Beef • Fresh water fish (roach, trout) • Salmon Pigeon (non-lead shot) • Quail • Deer (culled and pre checked for disease) • Marrow bones (as enrichment) • Knuckle bone • • Sardines (as enrichment) Pigs head (from human consumption

Table 2: Food items used by European collections for jaguars.

To promote species-appropriate hunting and feeding behaviours, food should be offered in a way that requires the cats to work for their food – for example hidden through the exhibit, hung in bags from trees or ropes, on branches or trees, or even buried. Providing enrichment opportunities on a randomised schedule will help to prevent the development of stereotypic behaviours. The institutional nutritionist or veterinarian should be consulted prior to introducing any food item for enrichment.

It is important NOT to present geriatric animals with feeding poles or high hanging food sacks etc. due to the likelihood of spinal arthritis.



Feeding enrichment examples at Chester Zoo.

2.2.3 Body Condition Scoring

Obesity is still seen commonly in managed jaguars, extreme body conditions are associated with increased health risks, poor reproductive performance and reduced longevity in domestic cats and dogs (Laflamme, 2005).

Animals should be weighed monthly to assess body condition and needs for diet changes. Depending on the build of the animal, even at a general ideal body weight for the species, it could be over or under weight, so it is also important to visually assess each animal - the most practical method for evaluating degree of fatness for animals which cannot be readily palpated is visual body condition scoring (BCS).

BCS systems provide a spectrum of fatness usually with 1 to 5 or 1 to 9 levels, for the jaguar care manual, AZA suggested a 9-point BCS scale, which is included in the document as Appendix 2. An advantage of a 9-point body condition scoring system is that scores of 4 (moderate low) and 6 (moderate high) serve as warning zones where diet or management changes can be made to avoid ever reaching body conditions of increased health risk (low 1–3 and high 7–9 scores). Additionally, a 9-point scale can be entered on animals' records in ZIMS.

2.2.4 Water



Fresh water should always be available. Watering devices built into the exhibit and enclosure may range from simple cement bowls to commercially available waterers. It is important to remember the jaguar's bite and body strength i.e., the watering device should be able to withstand the attention of a determined jaguar, remaining functional and avoiding injury to the animal. Pools may be used as drinking water sources, but should be designed to be easily emptied and cleaned.

Water sources must always be available.

2.3 Social Structure

Jaguars are generally solitary, with the usual exception of mothers and young offspring. However, accounts of wild jaguars living, and possibly hunting, together have been documented. Careful consideration should be given to ensure that animal group structures meet the social, physical, and psychological needs of jaguars, and the recommended social structure for managed jaguars is an adult pair (consisting of a male and a female), or a mother with immature offspring. Housing a single animal is also perfectly acceptable.

Following successful copulations, or at a time when pregnancy is suspected, a pair can be left together until two weeks before the suspected birth date. As with all cats, it is advisable to give the female as much privacy as possible when giving birth - thus the male should be kept as far away as possible. Generally, the female will not tolerate the presence of the male after the cubs are born, however temperaments among individuals vary a great deal, and in some cases family groups have been managed together successfully.

When wild jaguars are about one and a half years old, they have been observed to leave their mother. Zoo jaguars have been observed to be rejected by their mothers at less than one year old (however this may well be due to small enclosures and competition during feeding), while there are examples of offspring, or introduced, unrelated, animals successfully living together for many years.

2.3.1 Changing Group Structure

Sometime animals need to be moved between or within institutions, and thus may require introduction to new animals or reintroduction to their exhibit mates - it is important that all introductions are conducted in a manner that is safe for all animals and humans involved.

Most large felids are solitary in nature except during periods of breeding activity, therefore extreme caution and patience is recommended during introductions. Compatibility can be achieved through extended controlled introductions in a night house shift area, or adjacent enclosure, allowing auditory, olfactory, and visual contact, but preventing actual physical contact. An introduction schedule can be set up over a period of days or weeks to gradually reduce barriers until the animals are introduced into the same space. In case of aggression, it is recommended that items such as water hoses, air horns or CO_2 fire extinguishers, are to hand if needed to separate the cats safely.

When aiming to introduce a pair that are housed in adjacent enclosures without visual contact, olfactory contact can be provided by allowing each animal out into the exhibit on alternate days. This allows each cat to be aware and investigate the presence of another jaguar nearby. Introductions of males and females can be timed to coincide with oestrus in the female, which canbe seen through various indicators such as restlessness, increased vocalisations, neck-rubbing on items within the exhibit, the female rolling on her back, pacing, and the display of the lordosis posture (Stehlik, 1971).

During an introduction, multiple staff members should be present, but their presence should be low key so as not to add to the tension - they should be stationed discreetly around the exhibit. Although some aggression is normal, prolonged or intense physical aggression should be prevented - a time limit can be established for aggressive interactions before attempts are made to separate the cats. Aggression initiated by the female may be allowed for longer, as long as no serious injuries occur to the male. Females should be provided with access to the exhibit prior to the male being introduced.

When letting the male out, ensure the female is not near the door but facing it so that she knows when he is going to come out, and is thus not surprise by his presence, and doesn't allow herself to be stalked. Introduced jaguars should be monitored continuously throughout their first day together on exhibit.

2.3.2 Sharing Enclosure with Other Species

Jaguars are not recommended for mixed-species exhibits. Probably the most predictable variation in social behaviour will come when the female enters or leaves oestrus. During oestrus, males will likely show increased interest in the female, possibly behaving more aggressively, females also will show increased interest in the male. Mutual grooming and other non-aggressive physical contact may occur and lordosis and other postures by the female inviting copulation will become more frequent during the four-to-five-day oestrous period. Brief agonistic interactions are also normal, and the female should most often emerge as being dominant.

When housing jaguars in adjacent enclosures, extreme care should be taken, because animals will attempt to interact with each other, and unsupervised physical contact may result in either animal

being able to get hold of the limb, ear or some such part of the other jaguar. Once in possession, they are very difficult to get to disengage. It should also be noted that they are extremely strong and can bite through mesh as heavy as 3.75mm gauge chain link.

2.4 Breeding

Long-term assessment of ovarian hormones (oestrogen and progestogen) can allow female reproductive activity to be characterised. These can be analysed non-invasively by the collection of faecal samples. Such analysis (of faecal and urine levels of progesterone) can also be used to confirm pregnancy. Faecal analysis and monitoring of corticoids can also be particularly useful to monitor stress, especially if data on behavioural, environmental, and nutritional changes of the jaguar are also taken throughout the evaluation period.

From a case study at one collection in the UK, the first recorded mating of a pair (which had been together since 20 and 22 months old), occurred when the female was 2 years 10 months, and the male was 3 years old. This mating did not produce offspring. The second mating which produced a single cub, occurred when the female was 3 years and 6 months and the male was 3 years and 8 months.

2.4.1 Mating

If the male and female are separated before breeding, they should be introduced preferably in their den areas allowing auditory, olfactory, and visual contact, but preventing physical contact. Once the pair is put together, for the first few days copulation will take place 16-20 times a day; decreasing to three times a day at the end of the cycle. The average length of intercourse is 9 seconds with a range of 2-35 seconds. After one month of pregnancy vomiting can occur. After 3 months pregnancy will show a clearly swollen abdomen (Stehlik, 1971).

2.4.2 Pregnancy

The jaguar's gestation period is generally 91–111 days, and litter size is usually one to four cubs.

During gestation it is important to understand the physiological and behavioural changes that may occur. The physical appearance of a pregnant jaguar may not visibly change until very near parturition - if at all - and as a heavy-bodied cat normally, in some cases weight gain may simply not be apparent. The most reliable behavioural indicator of pregnancy is the absence of the monthly oestrus cycle, but this can be deceptive as the jaguar will only skip two cycles, prior to giving birth.

Like most carnivores, jaguars are secretive and this tendency is often intensified in late-term pregnancy, so pacing, agitation and attempts to hide or avoid interaction with keepers may be observed. Likewise, it is possible that a pregnant jaguar may become aggressive or, at the other end of the spectrum, appear to stare through people and animals unresponsively.

2.4.3 Birth

On the approach to parturition, staff should ensure the mother is comfortable in the area where birth will take place, and that this area is modified to ensure that there is nothing to cause possible harm to the cub. A consistent routine should be established, with one main keeper caring for the jaguars prior to the due date. Alternatively, two keepers assigned to the jaguar's care, provide alternate coverage to allow for days off or other absence. Bedding, for example hay or shavings can be introduced to allow the female to hide the cubs if sheis nervous, but the animals should be monitored for signs of any ingestion of bedding.

A den box, with at least five completely enclosed sides, should be constructed, with a threshold on the sixth side to prevent the cubs from immediately wandering. Ensure that the jaguars are not able to dismantle the box, and as with any enclosure, leave no sharp points or edges anywhere the animal might reach them. This should be done at least two days prior to the earliest due date to allow for acclimation.



Example of a den box for Amur leopard at Bellewaerde Zoo.

2.4.4 Development of Care and Young

Mother and cubs should not be disturbed for the first week after birth. It is recommended that staff should have video surveillance equipment / cameras / microphones to check the health of the cubs.

2.4.5 Hand-rearing

Although mothers may successfully give birth, there are times when they are not able to properly care for their offspring. This is particularly common in first-time mothers, in the wild and captivity, probably due to inexperience. Many of these females will go on to be successful the second time. Hand rearing is not recommended in the jaguar EEP because associated problems can lead to welfare issues, for example:

- social problems with other jaguars causing problems for introductions,
- inability to reproduce / provide maternal care / be a reproductively normal male,
- behavioural problems towards people potentially very dangerous,
- extreme aggression.

Jaguar mothers may not adequately care for their young due to various factors such as aggression, refusal to nurse, poor lactation, lack of attention or rejection, or medical issues, if necessary supplemental feeding of the cubs, with the cubs being returned to the mother afterwards, is possible. Jaguars are strongly individualistic, so no formula exists for such reintroductions. However, similar to most animal introductions, the animals should be acclimatised to the sight, smell and sounds of one another prior to any physical contact. Observation of responses to each other's presence (e.g. attempts to groom, relaxed or inquisitive postures versus bared teeth, hissing or paw- slapping) will be strong indicators of potential success. Such cases should be dealt with on a case-by-case basis. No data currently exists on the composition of jaguar milk, and only limited information is available on the composition of milk for other cat species.

2.4.6 Population Management

The goal of the jaguar EEP is to be self-sustaining, remaining stable at around 100 individuals. There are over 50 EAZA collections holding the species, and a number of non–EAZA and private collections who keep jaguars. It is agreed within the Felid TAG as part of the Regional Collection Plan that other more threatened big cat species should take priority in collections for space and investment.

For this reason, there is a non-breeding recommendation for most individuals in the EEP. However, to keep the population healthy there must be a small amount of breeding, so pairs that fulfil certain criteria will be recommended to breed and this will be re-assessed annually. Due to the high level of animals with unknown pedigree in the population, it is not possible to perform meaningful genetic analysis using PMx on the EEP population. Therefore, pairs are selected to maximise avoidance of inbreeding through pedigrees known. Collections with breeding recommendations will be contacted directly, and all collections who are recommended to breed are expected to be able to hold offspring for at least 24 months after birth.

2.5 Behavioural Enrichment

Jaguars are excellent climbers and swimmers, in the wild they patrol their home ranges frequently, and make use of vegetation as cover – thus exhibits designed to showcase those behaviours provide built-in environmental enrichment. Providing exhibit furnishings, and the ability to alter and change such furnishings to increase the complexity and enrichment opportunities (anchor points and reinforced attachment for large items, etc.) plays an important role in helping to improve psychological welfare.

Behavioural enrichment, refers to the practice of providing a variety of stimuli to an animal's environment, or changing the environment itself to increase physical activity, stimulate cognition, and

promote natural behaviours. Such stimuli can include natural and artificial objects, scents, and sounds. It is essential that stimuli are presented to the jaguar in a safe way to interact with, for example, food in a variety of ways, using the scent or sounds of other animals or species, and incorporating an animal training (husbandry or behavioural research) regime. Olfactory enrichment can encourage natural behaviours such as scent marking and exploratory behaviour. Extracts such as spices and perfumes, as well as different hunting scents and animal fur or feathers, can be used. Items from animals should always be frozen to remove parasites.

An enrichment programme for each jaguar should be developed, taking into account the natural history of the species, individual needs of the animals, and facility constraints. Each plan should include; goal setting, planning and approval process, implementation, documentation / record-keeping, evaluation, and subsequent programme refinement.

Institutions should have specific staff members who are assigned to oversee, implement, train, and coordinate enrichment programmes. Such staff should ensure that enrichment devices are jaguarsafe and integrated with veterinary care, nutrition, and animal training programmes to maximise the effectiveness and quality of care provided. Enrichment items should be presented on a variable schedule to prevent habituation. It is recommended that institutions develop a list of approved enrichment initiatives which is then available to and used by animal keepers.

It is important to note that even if items have been approved for use with jaguars at your collection, they should be reviewed when being used with new cats, and all enrichment items should be checked regularly to ensure an animals behaviour towards it hasn't changed. Below is a table of items used for enrichment by European collections, and in Appendix 3 more examples of enrichmentdevices and methods used at Chester Zoo can be found.

Table 3: List of possible enrichment items used for jaguars in European collections.

List of Food Items: Marrow bones, scatter feeds of normal diet, fish (frozen and fresh), crayfish, water melon, pumpkins, citrus (squeezed), blood trails, deer legs.

List of Novel Objects: Cardboard boxes, cardboard tubes, boomer balls (approved for big cats), cat toys such as tubes and donuts (approved for big cats), frozen blood lollies or ice balls (usually made in balloons with balloons removed after freezing), hessian sacks, hanging items (hessian rope), prey species faeces (pre checked for disease and parasites), fire hose toys or hammocks (warning as some individuals may try to eat firehose)

List of Olfactory Objects: Basil, paprika, chives, oregano, tea leaves (rubbed on enclosure furnishings), cinnamon (very popular with jaguars), garlic (rubbed on rocks and trees but cloves never left in in case they are ingested), perfumes (1 or 2 sprays max required and be mindful of alcohol content), fresh hay, fresh grass cuttings, citronella and neem oil diluted in water in spray bottle (natural fly repellent), citrus e.g. lemon, lime, orange (squeezed),

Below are a number of example enrichment devices from Chester Zoo, where an enrichment schedule was set up for use on a daily basis. This, combined with a few husbandry changes has shown to keep the jaguars stimulated and has reduced negative behaviours. The following account isgiven by Lead

Keeper, Rachael Bodenham:

"The schedule is made up of a variety of different ways we can feed our jaguars from manipulative/cognitive type enrichment (each of which are restricted to being used no more regularly than every two weeks), to more physically challenging methods which can be used as regularly as we like. Examples of the more effective cognitive enrichment include burying the food in the ground, scent trails with food hidden somewhere away from the trail and devices such asbamboo feeders and hollow logs. Burying the food encourages natural digging behaviours seen in their wild counterparts, we bury the food at least 0.5m deep and often we will dig out 'decoy' holes leaving food scents around them to try to extend the search time and effort required by the jaguar to locate and retrieve their food, which is also good for having active on-show jaguars for our visitors. Examples of the more effective physical enrichments include putting food up 6m tall winch feeder poles, proven to be highly beneficial in keeping captive big cats fit and healthy. Another is making useof the pool such as tying food to big floats or rafts, which encourages the jaguar to perform natural swimming and prey attack/manipulation in the water as well as dragging a very heavy item out from deep water. Another effective physical enrichment used regularly is the 'swing ball' device, this is where food is tied to a rope attached to a moveable eyebolt fixed in the top of a tall post, the food can be spun around the post creating a challenge to the jaguar in how to retrieve the food item.

Each time we feed our jaguars they are fed in a different way each time, this is ticked off on a list of enrichments which provides great communication within the team ensuring a good variety and avoids too much repetition. It does require some organisation and keeping a shed stocked up with enrichment items – which is always a great intern/volunteer task. But all in all it really isn't that time-consuming as has the flexibility to give them enrichments that are very quick to implement and when more time or bodies are available enrichments that are a little more challenging.

The schedule also comprises of a list of non-food related enrichments which can be provided if and when times available or even alongside food related enrichment. Some of the more effective non-food related enrichments include stuffed hessian sacks hung from height and hard objects such as logs wrapped in hessian the jaguar will use as scratch posts. Some of the more favoured sensory enrichments used include blood scent trails, dung from prey species and fresh herbs, favourites being cat mint, lavender and pine cuttings"

SWING BALL: Equipment and set up: (see pictures)

- 90 degree eye bolt, 3 nuts and 2 washers
- Tall post with a chamfered edge (e.g. pine), dug into the ground around 0.8m in depth using rocks and soil to secure it in place
- Short length of natural fibre rope preferably with a tiny eye splice at both ends to avoid fraying
- 2 D shackles one to attach rope to eye bolt and one for food to be attached
- Hole drilled directly down the centre of the chamfered edge end of post for the eye bolt with a diameter slightly wider so it can spin smoothly
- Hole into the side of the post using a hole saw drill bit, then drop eye bolt down central hole with a single washer and nut to put it in position, then with another washer and two nuts screwed onto the opposite end via the posts side hole to allow the bolt to spin but not work the nut off.
- N/B Much easier to put together on the ground



Set up and components of the swing ball enrichment device at Chester Zoo.

WRECKING BALL: Several hessian sacks folded around spliced end of rope, central sack filled with lavender.



Wrecking ball enrichment example at Chester Zoo.

RAIN MAKER: Bamboo pipe filled with pinecones and hessian sack sewn around the pipe and tied up with thick rope.



Rain maker enrichment example from Chester Zoo.

HOLLOW LOG: Natural heavy hollow log with several small holes with food item hidden inside and stuffed with leaves small logs etc.



Hollow log enrichment example from Chester Zoo.

2.6 Training

Institutions should use reinforcing conditioning techniques to facilitate husbandry procedures and if necessary behavioural research investigations. Animal training protocols and techniques should ensure that safety is prioritised for all involved. Below is a case study, given by Nicky Plaskitt, Animal Training Coordinator at Paradise Wildlife Park, highlights some of the benefits that can be seen from training jaguars:

"Across the world zoological facilities are realising the benefits of training many species to voluntarily participate in their own healthcare, particularly Category 1 species which can only be worked in protected contact.

Examples of such cooperative behaviours include hand injection (as opposed to darting) which can be used for routine vaccinations as well as administering prescribed medication, conscious blood draws and x rays, mouth examination, tooth brushing, eye and ear examination, and heart rate monitoring.

When these behaviours are trained using positive reinforcement and the animal is choosing to calmly offer the required behaviour, the results show less stress markers (cortisol levels in blood), and are more accurate (resting heart rate). This is useful for vets in determining baseline data for multiple species without putting the animal under anaesthetic.

Trained behaviours can also be used for preventative healthcare, or to monitor a recurring problem without the physical cost on the collection and the animal of repeated anaesthetics.

In addition to training behaviours saving money, and being safer for keepers and their animals, it canoffer cognitive stimulation and enrichment for the carnivores which can also be used to educate the public visiting your collection. We have seen very positive results from our collection, particularly the jaguars who are such an intelligent species they are very quick to learn what keepers are asking of them."

2.6.1 Crate Training

To direct an animal to enter and remain within an enclosed space, such as a shipping crate, regular practice under varying circumstances of noise and distraction is necessary. This can make it possible to crate and transport jaguars without having to anesthetise them. As with all training, attention should be given to security and safety for both animals and keepers.

2.7 Handling

2.7.1 Individual Identification and Sexing

Each jaguar must be individually identifiable and have a corresponding identification (ID) number.

Jaguars should be fitted with a microchip / transponder, in the supraspinatus or infraspinatus muscles either side of the spine of the scapula. Intramuscular implantation prevents migration of thechip and protects it from minor trauma. In the case of females, subcutaneous implantation in the scruff of the neck should be avoided due to vulnerability to damage during mating.

Every jaguar should be entered into an institution's animal records and given a local identifier at birth. All jaguars born in EAZA institutions will be entered into the EEP given studbook numbers.

2.7.2 General Handling

The jaguar is intelligent and strong, with lightning reflexes, thus sharing space with jaguars, whilst not under anaesthesia, is not recommended. Even protected contact should generally be avoided, and then conducted only when every possible measure to separate people from jaguar teeth and claws has been taken. In most instances, when working directly with large carnivores, it is recommended to involve more than one person, however safety assessments must be made by eachinstitution.

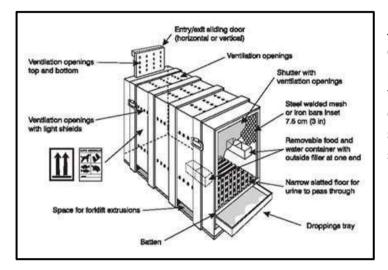
2.7.3 Catching / Restraining

Capturing, restraining and/or immobilising may be needed for normal / emergency procedures. It is important that all capture equipment must be in good working order and available to authorised and trained animal care staff at all times. Collections should develop and implement protocols for training staff members for safe capture and restraint of jaguars, which may be performed through a combination of training and chemical immobilisation. Training can allow basic visual checks, e.g. of foot pads, teeth, gums, tongue, and stomachs to occur without immobilisations, additionally, training may allow for blood draws and injections.

2.7.4 Transportation

Animal transportation must adhere to all laws, be safe and minimise risk to all animal(s), employees, and general public. Safe animal transport requires the use of appropriate equipment that is in good working order. Transportation documentation should always include copies of appropriate permits and authorisations, and if the animal is not owned by the shipping institution, permission must be gained from the owner well in advance of the move. Contact with the registrar, curator or proprietor of the owning institution to obtain permission and initiate relevant documentation should be made at least 30 days prior to setting a shipment date. The choice of whether jaguars should be shipped overland or by air rests on a variety of factors, including distance, season, climate and cost.

Jaguars should be shipped in separate transport crates; the height of the crate should allow the animal to stand up with its head extended and the length should permit the animal to lie in the prone position. A good rule of thumb is to allow 10cm clearance around the animal when standing. An interior height and width of 85cm by 85cm, and length of 185cm should accommodate the largest jaguar. The International Air Transport Association, (IATA), (International Air Transport Association, 2013/2014) specifies details of shipping containers, shown below. The equipment should provide adequate containment, life support, comfort, temperature control, food/water, and safety of the animal.



Jaguar transport crates should be constructed of hardwood, metal, plywood (or similar material) of no less than 1.3cm in thickness, welded mesh or iron bars. If wood is used, jaguar should not have direct accessto the surface as it could be chewed through.

Suggested design for a Jaguar transport container (IATA, 2013/14).

The frame should be made from solid wood or metal, bolted or screwed together. Welded aluminium crates which meet or exceed the IATA requirements are commercially available, or can becustom made. The frame should meet a spacer bar requirement of 2.5cm in depth to the sides for air circulation. If the weight of the container exceeds 60kg, or the animal is very aggressive, the frame should have additional metal re-enforcing braces. Suitable plywood or similar material should line the frame to give it a smooth and strong interior.

The floor should either be constructed in a narrow-slatted form over a liquid-proof tray so that all the excrement falls onto the tray, or it should be leak-proof and covered by sufficient absorbent material. The roof should be solid and a sliding or hinged entry/exit door should be provided. The front entry/exit door and the opposite end should be made of welded steel mesh or strong iron bars. It is recommended that a jaguar crate has doors at both ends.

Openings should be placed at heights that will provide ventilation at all levels. Exterior meshed ventilation openings with a minimum diameter of 2.5cm should be made on the sides, entry door(s), and roof. However, extreme care should be taken that transport crates have no spaces that allow jaguars to reach out with their claws, nor humans to read into the crate.

Food and water must be available during air transport, thus the crate must include food and water containers, which are fixed off the floor, to prevent soiling, near the front of the crate. There should be a safe way to access them from the outside for refilling water containers. Providing food to a jaguar during transport is not necessary if the transport takes less than a couple of days. It is recommended that water be provided at the beginning of transport and, when practical, during any stopovers.

Due to risk of injury to both animals and people, direct contact with jaguars during shipment should be minimised and crates should be constructed to provide safe visual access to the animal at all times. Unless conditions become life-threatening, standard practice is to maintain focus on reaching the destination once the animals are secured in shipping crates. Transport protocols should be well defined and clear to all animal care staff.

2.7.5 Safety

As a large predator with a powerful body, strong bite, and excellent jumping and ambush capability, the jaguar should be classified as an institution's most dangerous species regarding emergency escape and response procedures. Daily checks should be made of enclosure perimeters, and all doors and locks should be double checked with each use. Communication is extremely important keepers should have two-way radios, and ideally access to a telephone within the exhibit for additional communications when needed.

All emergency safely procedures must be documented clearly and made accessible to appropriate staff; they must be readily available in the event of an emergency.

Staff must be trained what to do in the event of an emergency escape, and such training must be recorded. Emergency drills should be conducted at least once a year, which also must be recorded, and evaluated to ensure that procedures are being followed and that staff training is effective.

Emergency protocols should include instructions for a clear communication system, and must include when and how local police or other emergency services are contacted. Such emergency policies can also be shared with local law enforcement and emergency officials.

If an animal attack occurs a written account outlining the cause of the incident, how it was handled, if injury occurred, and a description of any resulting changes to the safety procedures and exhibit must be prepared and kept on record. If a member of staff finds themselves in the same space as a jaguar, the following advice is given:

- Stay calm, talk to the jaguar, retreat slowly backwards (don't play dead, never turn and run cats like to chase things and hunting instinct may kick in if you turn and run).
- Keep looking as big as possible and if possible, maintain facing the cat, backing awayuntil you can get to a safe area.
- Slow blinking and chuffing may show non-threatening behaviour on your behalf but also may have no effect whatsoever depending on the individual cat - another option, depending on the individual cat would be to make yourself big and make as much noise as possible.
- If you can pick up anything that may be useful as a weapon or be used to defend yourself then do so slowly but try to avoid angering the cat if it is not attacking you e.g., don't throw things at it, big cats often react to aggressive acts with aggression so this could provoke an attack.
- Never block an escape route for the cat and try to avoid cornering it in any way.
- In the event of an aggressive encounter and a worst-case scenario where you may be under attack then very few options are available considering the power and weaponry of jaguars - try to cover your most vital areas e.g., neck and head as this is the most instinctive area the cat will try and attack, in some cases you can manipulate the head of a big cat by hooking inside the

cheek (this would mean putting your hand in its mouth which comes with its own risks) which mayprovide you with a few extra seconds to try and escape or injure the cat in another way.

A number of additional safety procedures are in place at Paradise Wildlife Park, examples of which are given below:

Two Person Check Protocol. There is a two person check protocol in place for:

- 1. Entering any area of a large carnivore enclosure (den, main enclosure, separation pen) lock the animals out of the area you need to enter then follow the Safe Lock-Checking Protocol below.
- 2. Allowing any large carnivore access back in to an area that you have serviced secure the area you have serviced then follow Safe Lock-Checking Protocol below.

The protocol requires two signed off large carnivore keepers, or one signed off large carnivore keeper and a senior keeper or animal park manager to double check that the area is safe and secure to enter for servicing or allow animals access after servicing. You will need the appropriate second person to do these checks with no exceptions.

Safe Lock-Checking Protocol

How to check an area is safe to enter or is secure for animal access.

- 1. Minimum of two staff required as stated in the Two Person Check Protocol above.
- 2. Both staff should acknowledge what they are about to check e.g. animal location, locks secured correctly, area they may want access to or give animal access to, etc.
- 3. Both keepers must visually check the animal's location and make sure they are in the correct area after the slide has been shut to lock them out of the required area e.g. the following steps
 - a. Keeper 1 shuts slide from tiger inside house to outside enclosure
 - b. Keeper 1 and 2 then go around and physically see all individual tigers locked out in the outside enclosure
 - c. Keeper 1 and 2 then both check locks on slide that was originally closed by Keeper 1
- 4. Both keepers must visually and physically check all bolts, padlocks and slides that are securing the animal or the area that is to be accessed (physically check means to pull on the padlocks and make sure they are locked correctly and are not faulty).
- 5. Once both keepers have completed the checks and have acknowledged to each other that they are satisfied that all checks are completed, only then can you continue the task at hand.

Hasp lock system

The lion, tiger and jaguar enclosures have been updated to facilitate a hasp lock system. This means there is an extra hole on certain slides for a multi lock hasp, on which individual keeper combination

padlocks are placed for extra security when entering an enclosure. This means these slides cannot be opened by anyone else while your own personal padlock is on a hasp. You must use the hasp system on all slides between yourself and the animals for this to be effective. When leaving the enclosure for any extended length of time while other staff members are still working in the enclosure (e.g., to deliver an experience) you should remove your own hasps in case other team members need to operate slides after you have left.

On occasion, staff or volunteers who do not have their own set of coded padlocks may be working alongside the large carnivore team, in this case a signed off large carnivore keeper will use their coded locks to secure an area for these team members and they are responsible for the people inside that area, they must ensure all people without coded locks have left the enclosure before removing their coded padlocks from the hasp.

The maintenance and gardening team will be issued with their own coded locks. The senior gardener or maintenance person conducting work in the enclosure will be responsible for their team members in that enclosure and should not remove their coded locks until all of their team members have finished and left the enclosure. In case of an emergency, bolt cutters are available in the capture cabin to remove locks. Never remove another keeper's locks unless under the instruction of a senior keeper or animal park manager in an emergency situation.



Hasp lock system used at Paradise Wildlife Park.

Emergency Flare Use

Emergency flares are provided in locked boxes at all enclosures. They can be carried by staff who have been trained in their use and have read and signed the Emergency Flare Protocol in the Animal Park Protocols and Policies folder. Additional flares can be found in locked boxes in the meat room and the gun room (gun room for use by trained fire arms team only). Emergency flares should be carried when entering any enclosure except for the Wolves and Ocelot.

Emergency Air Horn Use

Air horns are available across the section to be used as a distraction for cats in emergency situations.

Green Animal use only fire extinguishers

Green coloured animal-use-only CO2 fire extinguishers are available across the section to be used on catsin emergency situations. A map of where these are kept in the section is situated on the notice board in the gun room corridor.



Flares and green animal-only fire extinguishers at Paradise Wildlife Park.

2.8 Veterinary: Considerations for Health and Welfare

The following section is written by the jaguar EEP vet advisor, Melissa Nollet.

Veterinary services are a vital component of excellent animal care practices. A full-time staff veterinarian is recommended, however, in cases where this is not practical, a consulting/part-time veterinarian must be under contract to make at least twice monthly inspections of the animal collection and to attend any emergencies. Veterinary coverage must be available at all times so that any indications of disease, injury, or stress may be responded to in a timely manner.

Veterinary recordkeeping is an important element of animal care and ensures that information about individual animals and their treatment is always available. A designated staff member should be responsible for maintaining accurate animal veterinary record keeping. All pertinent health information for jaguars should be recorded per institutional protocols. As soon as the opportunity arises, it is recommended, although not a requirement, that institutions make full use of the Zoological Information Management System (ZIMS).

2.8.1 Preventative Medicine Plan

Institutions should have an extensive veterinary program that must emphasise disease prevention. Disease prevention is based on several pillars discussed below.

There is a call from the EAZA BioBank to collect extra blood, tissue and/or serum samples whenever an animal is sedated. This helps supporting population management and conservation research. The sampling protocol can be found in Appendix 4.

2.8.1.1 Routine examination

Jaguars should be examined prior to transfer, while in quarantine and thereafter on a routine schedule. The period between routine examinations should be based on the veterinarian's risk [of anaesthesia] – benefit [of gathering data on sub-clinical health concerns] evaluation, and the signalment/health status of the individual animal. When possible, examination under trained behaviour can give extra information on which to decide the need for anaesthesia. Regular routine examination, for example once every three years, can be valuable. In geriatric felids (>13y) a yearly examination is advised by the Felid TAG.

Routine examinations should include a whole-body exam (abscesses and lacerations), careful nail, and pad evaluation. Body weight, temperature, pulse, respiration should be recorded. Normal parameters include adult body weight (adult males 50-77 kg [130–180 lb.] and adult females 40-62kg [105–159 lb.]) (Species360, 2021), temperature (37–39.5 $^{\circ}$ C or 98.6–103. 1 $^{\circ}$ F), pulse (70–140 bpm), and respiration (8–24 bpm).

2.8.1.2 Oral examination

A thorough oral examination is an integral part of a physical examination. Dental issues being the 4th most common problem seen in Jaguars within the EAZA population (EEP survey 2020). The teeth and soft tissue structures of the mouth and throat should be examined for abnormalities. Dental tartar and calculi are common in managed jaguars. Tooth scaling and polishing should therefore be a routine part of any physical examination. If the cause of dental tartar and calculi is linked to food items offered, this must be addressed through review of diet. Particular attention should also be given to tooth fractures. They need to be treated when present and efforts need to be made to determine their cause, which should be removed where possible.

2.8.1.3 Clinical pathology

Clinical pathology is an important component of routine examinations. Laboratory tests that should be performed include:

- Bloods (see Appendix 5 for reference ranges)
 - Complete blood count (CBC)
 - o Biochemistry profile, including thyroid screening
- Faecal examination: (every 4 months)
 - Parasitology
 - o Bacteriology
- Specific testing on a case-to-case basis.

2.8.1.4 Medical imaging

Medical imaging should also be part of a routine examination. Thoracic and abdominal radiographs should be taken, and special attention should be given to evaluate the musculoskeletal system in older individuals (see Appendix 6 for advised protocol). Cardiac and abdominal ultrasound... should be performed when possible.

2.8.1.5 Pest control

An effective and safe method of controlling insects and rodents is mandated, and accurate records should be kept reflecting supervised, monthly, licensed pest control inspections and service. Rodent control can be achieved using snap traps, glue boards, and other non-chemical systems. Poison baits should be used only when there is no possibility of felid access to the bait or to treated rodents, resulting in secondary poisoning. Bait traps are highly effective, but should be kept dry, and be refilled at regular intervals. Insect control may include electronic insect killers, growth inhibitors, pest strips, and appropriate natural or synthetic insecticides. All chemicals should be approved by the institution's pest control personnel, including a member of the veterinary staff.

2.8.1.6 Vaccination

Vaccinations have been the mainstay of preventive medicine programs for both domestic and nondomestic cats. It is assumed that jaguars are susceptible to the common disease agents of domestic and non-domestic cats. Additionally, there is serologic evidence of infection with rabies, canine distemper, and feline immunodeficiency virus (Appel *et al*, 1994, Barr *et al*, 1989; Brown *et al*, 1993; Deem, 2001).

Clinicians should be familiar with the vaccines they administer and should only use killed or recombinant products, as modified live virus vaccines may cause vaccine-induced disease. Any adverse reactions should be reported to the EEP veterinary advisor so that this information can be disseminated to other institutions housing jaguars.

Vaccination with core vaccines, for protection against Feline Panleukopenia Virus (FPV), Feline Herpes Virus (FHV), Feline Calicivirus (FCV) should be routinely given to jaguars housed at EAZA institutions (Richards *et al*, 2006). A standard domestic cat dose of 1ml should be used in jaguars of all ages and vaccination interval should follow manufacturers guidelines. The use of other vaccines, including rabies vaccination, should be based on specific risk assessment. Currently the EAZA Felid TAG does NOT recommend the routine use of vaccines against canine distemper (CDV) and feline leukaemia (FeLV) in jaguars and other non-domestic felids.

In case of neonates the number of staff present and the associated sensory cues (smell, sound...) should be limited. They should receive their first vaccination at minimum 12 weeks of age, followed by a second injection 4 weeks later. If for any reason vaccination needs to be started earlier then a 3rd vaccine should be administered after 16w of age (Day *et al*, 2016). Thereafter a booster vaccination every 2-3 years should be adequate. This in the knowledge that there is species variation in immunologic response to vaccines, and thus vaccines may afford protection in one species but not another. The author knows of no experimental studies on the efficacy of vaccines in jaguars.

In recent years vaccine-induced neoplasia (Morrison *et al*, 2001) has become a concern in domestic cats and may be a concern in non-domestic felids (Larsen *et al*, 1998; Kinne and Tarello, 2007). The highest prevalence in domestic cats is linked to Rabies and/or Feline leukaemia (FeLV) vaccines (Saba, 2017). To minimize tumorigenesis associated with vaccination it is best to allow all vaccines to acclimate to room temperature before administration, to alternate the location where the vaccine is given (front and back, left and right) and to avoid the interscapular area. The use of less heavily adjuvanted vaccines (aluminium-based adjuvance) has also been advised, although definitive proof of cause is still absent in this case.

Vaccines should be used in conjunction with other preventive measures including limiting contact of managed jaguars with free roaming wildlife and feral carnivores (domestic cats and dogs), good hygienic standards, and maintaining adequate nutrition and overall health of jaguars to ensure strong immune systems.

2.8.2 Transfer Examination and Diagnostic Testing

The transfer of animals occurs following EEP recommendations, as part of a concerted effort to create a sustainable captive population. These transfers should be done as altruistically as possible and the costs associated with specific examination and diagnostic testing for determining the health of these animals should be considered.

Medical history should be sent to and reviewed by the receiving institution's veterinary staff prior to shipment.

If not already properly identified, the jaguar should be permanently identified with a transponder chip. The animal should be current on all recommended vaccinations (see above) which minimally should include panleukopenia (FPV), rhinotracheitis (FHV), and calicivirus (FCV).

Pre-shipment examination should include:

- Complete physical examination
- Bloods
 - Complete blood count (CBC)
 - o Biochemistry profile, including thyroid screening
- Faecal examination: 3-day pooled sample per individual advised
 - Parasitology
 - o Bacteriology
- Viral serology
 - o Calicivirus (FCV)
 - Canine distemper virus (CDV)
 - Feline immunodeficiency virus (FIV)
 - Feline corona virus (FCoV)
 - Feline leukaemia virus (FeLV)
 - Feline panleukopenia virus (FPV)
 - Feline herpesvirus (FHV; feline rhinotracheitis)
- Specific testing for
 - Dirofilaria immitis (PCR or snap-test)
 - Leptospira interrogans spp. (serology or snap-test)
 - Toxoplasma gondii (serology)
- Radiographs: thoracic and abdominal radiographs
- Ultrasound: examination of the reproductive tract is advised when animals are due to be placed in a breeding situation. This is to prevent unnecessary transports.

Food provided at the sending institution may be different from that which will be fed at the receiving institution and diets from the sending zoos should accompany the animal or at least be used initially by the receiving institution. It is imperative that the jaguar's diet not be switched immediately upon arrival at the new institution. It is better to slowly transition the jaguar to the newdiet while weaning it off the old diet. This will minimise possible anorexia and gastrointestinal problems that may occur with any dietary change. Most zoos will begin to transition the diet during the quarantine period.

2.8.3 Quarantine

Institutions must have holding facilities or procedures for the quarantine of newly arrived animals and isolation facilities or procedures for the treatment of sick/injured animals.

All quarantine, hospital, and isolation areas should follow EAZA standards/guidelines.

All quarantine procedures should be supervised by a veterinarian, formally written and available to staff working with quarantined animals.

If a specific quarantine facility is not present, then newly acquired animals should be kept separate from the established collection to prohibit physical contact, prevent disease transmission, and avoid aerosol and drainage contamination. Co-terminous quarantine may be necessary in some institutions.

Institutions must have zoonotic disease prevention procedures and training protocols established to minimize the risk of transferable diseases with all animals, including those newly acquired in quarantine. Keepers should be designated to care only for quarantined animals if possible. If keepers must care for both quarantined and resident animals of the same class, they should care for the quarantined animals only after caring for the resident animals. Care should be taken to ensure that these keepers are "decontaminated" before caring for the healthy resident animals again.

Equipment used to feed, care for, and enrich animals in quarantine should be used only with these animals. If this is not possible, then all items must be appropriately disinfected, as designated by the veterinarian supervising quarantine before use with resident animals. It is recommended that veterinarians at each institution develop their own specific disinfection protocols for animal management equipment and environmental enrichment provided in quarantine. These protocols should take into consideration the material to be disinfected and should ensure that disinfectants are thoroughly rinsed off before the equipment or enrichment is used again.

Quarantine should last a minimum of 30 days but avoid running longer than 45 days. (unless otherwise directed by the staff veterinarian). If additional carnivora are introduced into their corresponding quarantine areas, the minimum quarantine period should begin over again. However, the addition of mammals of a different order to those already in quarantine will not require the re- initiation of the quarantine period.

Jaguars are normally solitary so social issues should not arise from isolation during quarantine. However, some stereotypic behaviours have been seen in jaguars including pacing and tail sucking, occurrences which may arise in quarantine due to stress. Providing jaguars with environmental enrichment and space that allows them to exhibit species appropriate behaviours should be part of the quarantine protocols.

Medical records for each animal should be accurately maintained and easily available during the quarantine period. Three faecal samples for internal parasite evaluation should be submitted and all parasites treated appropriately while in quarantine. Usually between 14 to 21 days a quarantine examination should take place (e.g., allowing time for the jaguar to adjust to its new surroundings but also allowing enough time, prior to moving it to a permanent enclosure, to respond if a health issue is diagnosed during the exam). This also provides a good opportunity to satisfy all those requirements not met (if any) during the pre-shipment examination. Release from quarantine shouldbe contingent upon normal test results and should follow the protocols set forth by the staffveterinarian.

If an animal should die in quarantine, a necropsy should be performed, and the subsequent disposal of the body must be done in accordance with any national laws. Necropsies should include a detailed

external and internal gross morphological examination and representative tissue samples from the body organs should be submitted for histopathological examination (See Appendix 7).

2.8.4 Management of Diseases

Institutions should have an extensive veterinary program and should have the ability to isolate these animals in a hospital setting for treatment if necessary. Hospital facilities for jaguars should have medical imaging equipment or access to such services, contain appropriate equipment and supplies on hand for treatment of diseases, disorders, or injuries. Staff trained to address health issues, manage short- and long-term medical treatments and control for zoonotic disease transmission should be available.

Jaguar keepers should be trained for meeting the animal's dietary, husbandry, and enrichment needs. Non-domestic felids may hide signs of illness until a disease is advanced. In managed settings, it is important that animal care staff be astute to subtle changes in behaviour or physiologic signs that may suggest illness. Keepers that have daily contact with jaguars are often the best placed for noting these subtle changes. Any change in appetite, urination, defecation, or general behaviour should be documented. For example, changes in urine and faecal colour, quantity and consistency should be noted. Other visual observations that can be obtained from outside the enclosure include lameness, evaluation of breathing pattern and rate (N=8-24 bpm). Other physiologic parameters, such as temperature (N=37–39.50 °C or 98.6–103.10 °F) and pulse (N=70–140 bpm) require handling the animal. Jaguar keepers noticing signs of illness should follow the protocol set forth by their institution for proper reporting of health concerns.

2.8.4.1 Non-infectious diseases

There are several diseases documented in the literature that occur in managed jaguars. Two retrospective studies on the morbidity and/or mortality of jaguars in AZA zoos from 1982–2002 (Hope and Deem, 2006) and in EAZA zoos from 1998-2018 (Duque-Correa, 2019) provide a good review of these diseases. Non-infectious diseases include a high incidence of neoplasia which may bespecies related but also associated with husbandry under zoo management and/or longevity. Dental disease, including calculi and tooth fractures, is commonly seen in managed jaguars. Laceration, withor without subsequent abscess formation, can also be a problem. Renal and musculoskeletal diseases occur in jaguars, as in other large cat species, especially as they age.

A recent study looking at neoplasia's in zoo managed felids reported that the reproductive system was most affected, followed by the haematolymphoid system and the respiratory tract in third position. Another finding was that *Panthera*-species have a higher likelihood of developing neoplasia's (5x), a higher probability of malignancy and a higher prevalence of neoplasia in the reproductive system (Moresco *et al*, 2020). Species-specific the latter was highest for jaguars (20%) and might be linked to BRCA1 mutations. These mutations were traced back to common ancestors within the North American zoo population (Corner *et al*, 2017) and malignant ovarian neoplasia's weren't a noticeable feature outside of the North American jaguar population.

Within the EAZA population reproductive disease was only reported in geriatric females (>16y) and of these 71% was neoplastic in origin. Reproductive disease in males is far less common (Duque-Correa, 2019).

2.8.4.2 Infectious diseases

Jaguars are likely susceptible to the same pathogens as other members of the *Felidae* family (Fowler 1986). Many infectious agents have been documented to cause morbidity and/or mortality in jaguars. Several are briefly discussed below, however this is by no means a limited list.

2.8.4.3 Viral diseases

<u>Rabies</u>

Rabies is a highly fatal member of the rhabdovirus family requiring direct contact for transmission. All warm-blooded animals are susceptible to clinical rabies disease. In Europe some countries have been declared free of rabies, linked to vaccination campaigns in wild foxes. In other countries rabies is still present in wildlife (fox, raccoon dog, bats) and the reintroduction of rabies in currently negative countries is a viable risk (Ex. Germany 2005). Vaccination is advised and might also be a legal obligation in endemic areas or for animals due to transfer to these regions. In countries officially free of rabies, the decision to vaccinate or not is left at the discretion of the zoo's veterinarian.

Canine distemper virus (CDV)

CDV has been reported in all families of terrestrial carnivores. Since 1991, CDV infections have been reported in five species of free-ranging and *ex situ* felids from at least eight discontiguous sites and epidemics in managed lions, tigers, leopards, and jaguars have been reported in the 1990s asreviewed in Deem *et al*, 2000. CDV-neutralising antibodies have been detected in free-rangingjaguars (Furtado *et al*, 2008) and there is some indication that large felids might be part of natural host spectrum for CDV (Terio and Craft, 2013). The EAZA Felid TAG does not currently recommend vaccinating managed non-domestic felids. However, in the USA, the new canarypox-vectored CDV vaccine (e.g., PUREVAX Ferret distemper vaccin[®], Merial) has proven safe and effective in many non- domestic species (Georoff *et al*, 2020). Some zoos are currently using this vaccine in jaguars and other non-domestic felids in the face of CDV epidemics. It isn't licensed in the EU and is therefore subject to individual import licenses and requires a genetically modified organism containment order for the collection where it will be used.

Feline leukaemia virus (FeLV)

FeLV is a major pathogen in domestic cats (associated with neoplastic and non-neoplastic disease) throughout the world. Direct contact with an infected individual or transfer through lactation is required for infection to take place. Reliable testing (e.g., Witness[®], Zoetis) and vaccination have, in the last years, reduced prevalence in domestic cats. Infection and resulting disease are rare in non-domestic felids. Even though a killed vaccine (e.g., Versifel FeLV[®], Zoetis) is available for use in domestic cats it is NOT recommended for use in non-domestic felids at this time.

Feline immunodeficiency virus (FIV)

FIV is an often-fatal disease of domestic cats caused by a lentivirus. However, there is no clear correlation between virus infection and disease in non-domestic felids. No vaccine is available against this disease. Since there is insufficient data on the epidemiology and pathogenicity of FIV in jaguars, it is important to test all managed jaguars for FIV-specific antibodies. A good time for this is before each transfer out of an institution. If an animal is seropositive it is imperative that the EEP coordinator is notified.

Feline Herpes Virus (FHV)

Feline herpes virus, also known as feline rhinotracheitis virus, is shed in ocular and nasal secretions as well as in saliva. It's highly contagious and clinical signs most often involve the upper respiratory system. Often self-limiting, acute infection can however cause death in neonatal cubs. Infected animals become latent carriers and the virus can reactivate when the animal is stressed (transfer, parturition...). Core vaccines include FHV and whilst it does not protect against infection it does reduce clinical signs, viral shedding and prevents reactivation. It is imperative to know if female jaguars in a breeding situation are carriers. If they are then an adjusted vaccination schedule is advised to protect the cubs: use an attenuated vaccine, instead of killed, start vaccination at 5w followed by 3 further injections on week 7, 9 and 12 weeks.

Feline Panleukopenia Virus (FPV)

Feline panleukopenia virus, also known as feline parvovirus, is transmitted by direct and indirect contact between individuals. The virus can be shed up to 6w in faeces post recovery and it is also very persistent in the environment. Illness can vary from subclinical, to fever, vomiting, diarrhoea.... In individuals below 5 months mortality is high. FPV is included in the core vaccinations.

Feline Corona Virus (FcoV)

Two forms exist: Feline enteric corona virus (FeCV) which affects the intestines and Feline infectious peritonitis (FIP). FeCV is highly contagious between cats. Infection can be asymptomatic or cause of mild diarrheoa with symptoms lasting for up to five days. FIP is a fatal mutant variant of the standard FeCV that causes severe disease in both domestic and non-domestic cats. Mutation occurs within the individual. A 'wet' and a 'dry' form exist, the former causes transudate effusions, the latter leads to the formation of (pyo)granulomatous lesions and has a slower onset with often vague symptoms. There is a commercial modified live virus vaccine (Primucell FIP®, Zoetis) marketed for domestic cats. Presently, this vaccine is NOT recommended for use in non-domestic felids due to questions about its safety and efficacy.

SARS-Cov-2 Virus

SARS-Cov-2 (COVID 19), is a very new virus affecting the human population. A lot is still unknown; however, disease has been observed in domestic and wild felids (lion, tiger, leopard). Symptoms include mild respiratory signs, but asymptomatic wild felids have also tested positive (rRT-PCR). For more in-depth information please consult the EAZWV Transmissible Diseases Handbook.

2.8.4.4 Bacterial diseases

Leptospirosis

Leptospirosis, caused by a variety of *Leptospira interrogans* serotypes, has so far been reported in over 150 mammalian species, including humans. There are many reports of significant morbidity and mortality in zoo collections. The disease does not appear to be a major problem in felid species, but it has been linked to interstitial nefritis (Arbour *et al*, 2012). Cats have been shown to shed *Leptospira* which, as a zoonosis, also needs to be taken into consideration when working with in non-domestic felids (Dorsch *et al*, 2020). Several killed vaccines containing 3 serotypes are licensedin Europe for canine use. Some have been used off license in *ex situ* felids during leptospirosis epidemics. Unfortunately, limitations to this vaccine are the lack of cross protection for one serotype when vaccinated with a different serotype and the short lived (two to three months) immunity post-vaccination. Standard vaccination is therefore NOT advised in ex-situ felids.

Bacillus anthracis

Bacillus anthracis, a gram-positive bacterium, has been reported in carnivores worldwide, including most Felidae. For jaguars only one case report can be found attributing an acute death to a *B. anthracis* infection (Abdulla *et al*, 1982). Infection occurs percutaneously, through inhalation or ingestion of spores. These produce toxins and cause a septicaemia affecting multiple systems which can lead to acute death (Terio *et al*, 2018). When an animal is found death with blood visible around orifices, swollen abdomen, or head it's imperative the body isn't opened. This to prevent the spread of the bacteria. *B. anthracis* is not endemic in Europe, but still quite common in the Mediterranean area. In affected areas vaccination is advised.

<u>Ehrlichia</u>

Ehrlichia species are mostly transmitted by ticks and occur in Southern Europe. Wild jaguars were found seropositive for *Ehrlichia canis* (Widmer *et al*, 2011). So far, no clinical signs have been described in jaguars. In domestic cats' clinical signs vary from fever, lethargy, loss of appetite, to a decreased production of platelets and related clotting problems, pale mucous membranes, dyspnoea... (Little, 2010).

Chlamydophila felis

Chlamydophila felis is the causative agent of acute or chronic conjunctivitis, and pneumonia in domestic cats. Vaccination in domestic cats is only advised to help control the spread of the bacterium in multiple cat environments where verified infections have occurred. A multivalent vaccine containing inactivated *Chlamydophila felis* (e.g., Fevaxyn Quatrifel[®], Zoetis) is available for use in domestic cats, however standard vaccination in non-domestic felids is NOT recommended at this time.

2.8.4.5 Ectoparasites

Not many reports exist on the prevalence of ectoparasites in jaguars. Fleas and ticks have been found in wild jaguars (Durden *et al*, 2006). However, as with most infectious agents it is assumed that they are susceptible to those ectoparasites affecting domestic cats. When required, treatments (product and dosages) are also based on domestic cat medicine.

2.8.4.6 Endoparasites

Several endoparasites are known to infest jaguars. Regular faecal examinations should be performed, and action should be taken according to the results (presence or absence). Do rememberthat a negative result does not automatically translate to the absence of parasites, as latency periods and intermittent shedding might be involved. Appropriate treatment should only be given following a positive result, this to prevent parasite resistance. In the case of a highly contaminated enclosure, or environment where re-infection risk is high, blind treatment at set intervals might be required.

In the wild 64% of faecal samples were positive for at least one parasite. Nematodes were the most prominent group with 50% (Solórzano-garcía *et al*, 2017). Faecal analysis of captive EEP jaguars most often gave a negative result for both parasites and bacteria, so no treatment was required. When parasites were found, nematodes of the families Ascarididae and Strongylidae were most often detected, followed by other nematodes, including Toxacaridae. Third came *Coccidia*. Clinical signs were only reported in a case of coccidiosis and one infection with *Helicobacter* (EEP survey 2020).

Drug	Parasite class	Application	Dosage
Pyrantel	Nematodes	PO	3-5 mg/kg SID for 3-5 days.
Fenbendazole	Nematodes, cestodes	PO	10mg/kg SID for 3-5 days
	Giardia	PO	50mg/kg SID 10-14 days
Febantel*	Giardia	PO	56 mg/kg SID 5 days
lvermectin	Nematodes	SC / PO	0,2 mg/kg single application 0,1-0,2 mg/kg monthly for <i>Dirofilaria</i> prophylaxis
Praziquantel	Cestodes	SC / PO	5,5-6,6 mg/kg single application
Milbemycine oxime	Nematodes	PO	2mg/kg single application 2mg/kg monthly for <i>Dirofilaria</i> prophylaxis
Moxidectine	Nematodes	Topical	1mg/kg monthly for <i>Dirofilaria</i> prophylaxis**
Sulfadimethoxine	Coccidia	PO	50mg/kg SID for 10-14 days***
Toltrazuril (oral formulations)	Coccidia	PO	30mg/kg SID: 3 days on, 4 days off, 3 days on****

Table 4: Drugs to treat jaguars for endoparasites

* combo preparations to be used based on febantel contents

** combo preparations to be used based on moxidectine contents (Arther *et al*, 2005). Watch with canine products, moxidectine overall 1/4th of that in a feline product!

*** (Plumb, 2011)

**** off label use in domestic cats (Hirose *et al*, 2015)

<u>Toxoplasma gondii</u>

Toxoplasma gondii is a protozoan parasite which can infect most warm-blooded animals through ingestion of sporulated cysts, including humans. Felids are the definitive host for this parasite and infection in domestic cats is generally asymptomatic. Symptoms are most often seen in immunodeficient animals. Free ranging jaguars have been reported as seropositive for *T. gondii*, but no clinical signs were noted (Furtado *et al*, 2008).

Toxacara and Toxascaris

Toxacara cati and *Toxascaris leonina* are common nematode parasites of domestic cats, which are also regularly found in wild felids, including jaguars. Infection occurs by ingestion of the parasitic egg either direct or its paratenic host (rodents). Often a coinfection is present with both parasites. Eggs can survive in a damp and cold climate for months, but have difficulty surviving in a dry and warm environment. Treatment is possible and often effective, but reinfection is frequent (Okulewicz *et al*, 2012). Both parasites have a zoonotic potential.

Aelurostrongylus

The common cat lungworm, *Aelurostrongylus abstrusus*, has been shown to infect wild felids (Di Cesare *et al*, 2016). Larval stages can stay infective in cat faeces for up to 5 months after which a gastropod is needed as intermediate host. The L3 larvae incapsulate within the host and stay infective for up to 2 years. Direct ingestion of the gastropod or of a paratenic host causes infection of the cat. In felids the parasite migrates to the lungs where it matures.

Symptoms, when they occur, are very similar to those of infection with *Dirofilaria* or *Capillaria aerophilus*: coughing, dyspnea, hypertension, eosinophilia on CBC.... Detection is not easy. Radiographic and CBC changes are non-specific, serology can't differentiate between a historic or active infection (Hubers, 2014). Larvae can be detected in faecal samples using a Baermann flotation, however it's important that the sample is not contaminated with free living nematodes (Conboy, 2009). The gold standard is PCR on faeces or the Baermann supernatans.

<u>Dirofilaria</u>

Several *Dirofilaria* species are known to affect mammals all over the world. This parasite requires a mosquito as intermediate host and vector. In the new world 3 species are known: *Dirofilaria tenuis* which affects raccoons, *D. ursi* affects north American species, mainly bears and *D. Striata*, a parasite of wild felids in North, Central, and South America.

D. repens is exclusive to the old world and causes subcutaneous dirofilariosis in carnivores and humans.

The most widespread is *D. immitis*, better known as heartworm and has been observed in free- ranging jaguars (Otto, 1974). It's prevalent in warmer areas across the world, where transmission, by mosquitoes of the Culicidae family, is possible throughout the year. The incubation period is 5-6 months and symptoms vary from cough and listlessness to sudden death. An antigen ELISA is available, unfortunately, in felids, a lot of false negatives occur. When heartworm is suspected thoracic radiographs can give supportive evidence, but cardiac ultrasound is the best diagnostic option in cats. Treatment of adult worms isn't without risk and in felids only supportive treatment is

advised. Prevention is the way to go in endemic areas. The medications target the early larval stages (L1-L4) and therefore need to be administered regularly. (Payne *et al*, 2014)

2.8.5 Health Issues in Aging Jaguars

Certain disease processes, some of which are highlighted below, have a higher prevalence in older individuals. The felid TAG therefore advises to perform a health check in felids over 13y old and, depending on clinical findings, yearly or 3 yearly thereafter. Do be aware that anaesthesia in older individuals might not be as straight forward and if possible, a blood sample for pre-anaesthetic evaluation should be taken.

Renal disease is not infrequent in old felids (Junginger *et al*, 2015; Newkirk *et al*, 2011, Hope and Deem, 2006). Polyuria (increased urination), polydipsia (increased drinking) and halitosis (bad breath) can be noted in affected individuals. Urine sampling can give an indication. However, diagnosis requires a blood sample. The condition is unfortunately fatal, so treatment is limited to supportive care (oral medication, dietary changes).

A current study is looking into degenerative joint disease in aging large felids. Arthrosis of elbow, shoulder, hip, and stifle joints is not uncommon, spondylosis is also a frequent finding. Clinically a cat might become slower, less likely to jump or climb. When radiographs are planned as part of a workup, it would be appreciated if the protocol in appendix 8 can be followed. Resulting images can then more easily be compared and included in the study. Radiographic images can always be forwarded to the EEP vet advisor.

Dental disease occurs in all age classes, and dentition should therefore always be looked at during an examination. If possible, on trained behaviour so a treatment plan can be made up before anaesthesia. Broken, chipped, split teeth, gingivitis, worn down teeth... are all possible presentations and can give serious discomfort and lead to tooth root abscesses, anorexia, depression, salivation....

Neoplasia was the cause of mortality (natural or euthanasia) in a third of the deaths of geriatric jaguars within the EAZA population. In adult jaguars this was half (15%), so neoplasia needs to be a differential whenever an older jaguar presents with any clinical signs.

2.8.6 Contraception, reproductive evaluation, and artificial insemination

Thanks to EAZA RMG for providing the following data.

2.8.6.1 Contraception

Separation of the sexes

Separation of the sexes is not recommended for carnivores due to the increased risk of reproductive failure and uterine pathology in cheetahs (Crosier *et al*, 2011) and canids (Asa *et al*, 2014). Similar research has not been conducted in jaguars, but the effects of separation may be similar.

Surgical sterilization; male

Castration (recommended): Permanent sterilisation by surgical gonadectomy. This procedure should not be carried out in individuals who are likely to receive breeding recommendations. As the testes are removed, testosterone mediated aggression may decrease. Males may also experience a decrease in body size and testicle size. If pre-pubescent males are castrated, they may become taller due to the delay in the closure of the epiphyseal plates.

Vasectomy (not recommended): Vasectomies are not recommended in this species due to the risk of females developing reproductive pathologies as a result of repeated oestrus cycles and induced ovulations through mating but without conceiving.

Surgical sterilization; female

Ovariohysterectomy: For individual jaguars unlikely to receive a breeding recommendation, permanent sterilisation by surgical ovariohysterectomy for females. Removal of the uterus as well as ovaries is preferable in older females, due to the increased likelihood of uterine pathology with age.

Ovariectomy: Removal of ovaries is a safe and effective method to prevent reproduction for animals that are eligible for permanent sterilization. In general, ovariectomy is sufficient in young females.

Tubal ligation (not recommended): Tubal ligation will not prevent the potential adverse effects to females that can result from prolonged, cyclic exposure to the endogenous progesterone associated with the pseudo-pregnancy that follows ovulation induced by copulation.

Hormonal contraception; male and female

GnRH agonists (recommended): GnRH agonists, such as deslorelin (Suprelorin; guideline below) or leuprolide acetate (Lupron), reversibly suppress the reproductive endocrine system, preventing production of pituitary hormones (follicle stimulating hormones, luteinizing hormone) and gonadal hormones (estradiol and progesterone in females, testosterone in males). The observed effects are similar to those following either ovariectomy in females or castration in males but are reversible. GnRH agonists first stimulate the reproductive system, which can result in oestrus and ovulation in females or temporary enhancement of testosterone and semen production in males. The stimulation phase must be suppressed in females to prevent the sustained elevation of progesteronefollowing ovulation by treatment with an oral progestogen (Megestrol acetate; Megace®/Ovaban®/Ovarid®) for 7 days before and 8 days after implant insertion (Wright *et al*, 2001). The risk of developing uterine pathologies following the 15-day treatment is much reduced compared to the 3-4 week stimulation phase. The stimulation phase cannot be suppressed in males.

Deslorelin acetate (Suprelorin[®] - subcutaneous implant): In males, 1 x 4.7mg implants are recommended for a minimum duration of 6 months and 1 x 9.4mg are recommended for a minimum duration of one year. In females, 2 x 4.7mg implants are recommended for a minimum duration of 6 months and 2 x 9.4mg are recommended for a minimum duration of one year. Due to the initial stimulation of the reproductive system, the first bout in females must also be supplemented with additional contraception e.g. oral megestrol acetate (Ovarid[®]/Megace[®]) daily 7 days before and 8 days after placing the implants. In males, the stimulation period cannot be

suppressed therefore additional contraception should be used in females or the sexes should be separated for 6-8 weeks after implant placement, to allow for viable sperm to clear from his system. Suprelorin is designed to be fully reversible although time to reversal is variable in felids. To increase the chances of a full reversal, place the implant in such a way that facilitates removal e.g. in the umbilical region, inner thigh, armpit.

Suprelorin should not be used during pregnancy since it may cause spontaneous abortion or prevent mammary development necessary for lactation. It may prevent initiation of lactation by inhibiting progesterone secretion, but effects on established lactation are less likely. New data from domestic cats have shown no effect on subsequent reproduction when treatment began before puberty.

Suprelorin is more commonly used in females because monitoring efficacy in females by suppression of oestrous behaviour or gonadal steroids in faeces is easier than ensuring continued absence of sperm in males.

Hormonal contraception; female only

Progestin-based contraceptives (e.g. Implanon[®]/Nexplanon[®], Depo-Provera[®], Delvosteron[®], Jadelle[®]): Progestin-based contraceptives are not recommended for use in female jaguars due to the increased risk of mammary and uterine pathologies of elevated levels of circulating progestogens without a resulting pregnancy.

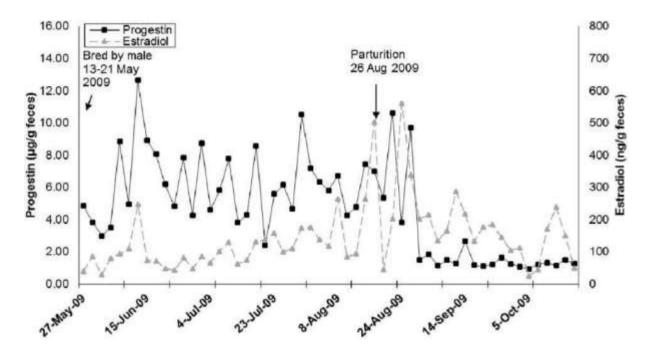
2.8.6.2 Assisted reproductive techniques

Reproductive hormone monitoring

Reproductive hormones can non-invasively be monitored through faecal sampling. For males, faecal samples should be collected 1-3 times per week, while faecal samples should be collected 3-4 times per week for females.

In wild jaguar males, faecal androgens are significantly higher in the wet season than in the dry season (Morato *et al*, 2001).

In jaguar females, behavioural oestrus can be characterised by rolling, disinterest in food, vulvar swelling, discharge, and lordosis (Barnes *et al*, 2016). Hormonal oestrus in Jaguar females was classified when estradiol concentrations were greater than the baseline + 1 standard deviation (SD) in the study by Barnes *et al*, (2016). Baseline concentrations are calculated by using an iterative process in which values that exceed the mean + 1.5 SD are excluded until no values exceed the mean + 1.5 SD (Brown *et al*, 2001). Hormonal oestrus lasts for 6.5 ± 0.3 days (mean \pm SD), while behavioural oestrus lasts for 7.8 ± 0.7 days (Barnes *et al*, 2016). Pregnancy and pseudopregnancy can be characterized by monitoring faecal progesterone concentrations. However, it may be difficult to discriminate between conception and non-conceptive luteal phases (pseudopregnancy) by progesterone only. Baseline concentrations are calculated as for estradiol. Luteal progesterone concentrations are those greater than the baseline + 2 SD (Barnes *et al*, 2016). Pseudopregnancies are classified when progesterone concentrations return to baseline following a sustained rise of > 20 days (Barnes *et al*, 2016). Pregnancy lasts approximately 98 days in jaguars (Figure 1).



Endocrine profile for a pregnant jaguar following a natural mating. Figure taken from Barnes *et al,* (2016).

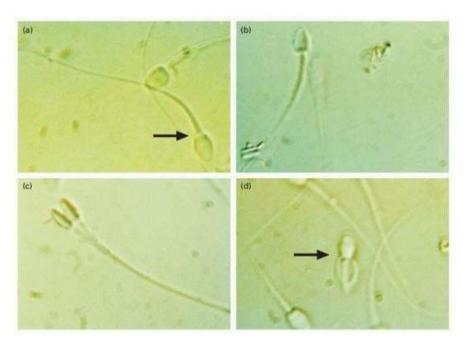
Understanding ovarian activity is essential for the successful application of assisted reproductive techniques (Pelican *et al*, 2006).

Fertility evaluation and gamete recovery; male

Semen collection under anaesthesia is an accurate method of gaining information on male fertility. Each testis should be measured and a combined testicular volume should be calculated (Howard, 1993). Reference values for captive and wild jaguars can be found in Morato *et al*, (2001). The penis should be extruded from its sheath and examined for the presence of spines (scale of 1-3, 3 = most prominent spines (Morato *et al*, 2001; Swanson *et al*, 1995). Sperm can be collected by electro ejaculation (Morato *et al*, 1999, 2001) or urethral catheterization (Ribeiro de Araujo *et al*, 2018) and should be evaluated by measuring: 1) semen volume, semen concentration (sperm count), total motility and forward progressive motility; 2) morphology: proportions of normal and abnormal sperm forms (Figure 2), all via phase microscopy (630x) (Howard *et al*, 1990); and 3) acrosomal integrity using specific stains, such as the rose bengal/fast green stain (Pope *et al*, 1991). Reference values for captive and wild jaguars can be found in Morato *et al*, (2001). Semen is usually very dilute (~5 x 10⁶/ml) but large volumes are generally recovered (up to 20 ml) (Morato *et al*, 2001; Swanson *et al*, 1995). A high percentage of abnormal sperm morphology is common in feline species, and in jaguars reported to be up to 65% in captive jaguars and 51% in wild jaguars (Morato *et al*, 2001).

Fine needle aspiration of the testes/epidymidiscan be used to evaluate sperm production if semen collection is not possible (Leme et al, 2018).

Semen cryopreservation has been performed successfully in various feline species, one applicable method is described by Swanson et al (2003). Reported survival following cryopreservation is relatively good (Swanson et al, 1996), with sperm retaining 25-50% motility post-thaw (Graham et al,1978), depending on initial quality of the sample.



Morphology of jaguar spermatozoa.

a) normal morphology,
b) malformed head shape,
c) biflagellate,
d) tightly coiled flagellum.
Figure taken from Morato *et al*, (2001).

Fertility evaluation and gamete recovery; female

Hormonal monitoring is encouraged to determine whether females are reproductively active. From live animals, the successful recovery of oocytes has been described in Morato *et al*, (2000).

From deceased animals, the collection of oocytes can be accomplished 8 hours after death ovary dissection and mechanical follicle isolation (Jewgenow *et al*, 1997). Preantral follicles can also be collected from deceased animals, following methods in Jewgenow and Stolte (1996) or Jewgenow *et al*, (1997), and matured *in vitro*.

Ovarian stimulation: The success of assisted reproductive techniques (ARTs) such as *in vitro* fertilization (IVF) or artificial insemination (AI) in felids is generally low. Oestrus induction via exogenous hormones is desired to have timed and planned procedures and several protocols for felids are published (Brown *et al*, 1995; Goeritz *et al*, 2012; Howard *et al*, 1992; Kutzler, 2007; Pelican *et al*, 2006), however, the overall success rate of these protocols is limited. Since jaguars express overt signs of heat, natural oestrus AI may be a promising option.

Ovulation induction: In jaguars, ovulation during natural heat can be induced through using exogenous gonadotropins (protocols described in Barnes *et al*, 2016), but can in some cases be induced by physical vaginal stimulation (Barnes *et al*, 2016). Ovulation in exotic felids tends to occur approximately 37-42 hours after treatment with gonadotropins (Pelican *et al*, 2006). The most common exogenous hormone treatment for ovulation induction reported is human Choriogonadotropin (hCG) or Gonadotropin Releasing Hormone (GnRH) (Callealta *et al*, 2019).

Artificial insemination (AI): Anecdotally, successful AI has been achieved in jaguars; however, exact techniques have not been published. Successful AI protocols have been published for several feline species. One should distinguish between non-surgical and surgical AI options. Successful, non- surgical AI during natural heat has been repeatedly performed in Asiatic golden cats, Persian leopards and African lion (Callealta *et al*, 2019; Dresser *et al*, 1982; Lueders *et al*, 2014, 2015).

The most reported AI method for smaller felids is the laparoscopic approach, where the semen sample is placed into the oviduct or uterine horn under endoscopic abdominal view.

In vitro fertilization: Techniques have been described in Morato et al, (2000).

2.8.7 Anaesthesia

Whenever a veterinary procedure is required on a jaguar, planned or in emergency, chemical immobilisation is necessary. As zoo vets most of us haven't got extensive knowledge or experience of an anaesthetist. It is therefore important to really understand the medications used (pharmacodynamics, dosages, side effects...). Developing safe and effective protocols and checklists can make an anaesthesia more manageable. Remember: *"There are no safe anaesthetic agents, there are no safe anaesthetic procedures. There are only safe anaesthetists."* -Robert Smith, MD.

2.8.7.1 Preanaesthetic evaluation and preparation

It is important to consider the patients' health status. Since anaesthetic agents cause cardiopulmonary depression, pre-existing illness might affect to some degree the patient tolerance to this cardiovascular disturbance. The protocol should be adapted as and when required: neonates versus geriatric animals, known or suspected underlying conditions, planned procedure or emergency (shock...). If possible, collect a blood sample through positive reinforcement training. Evaluation of biochemistry and CBC should be done before any planned procedure.

Preanaesthetic fasting is done to ensure the cat's stomach is empty. Aside from time, stomach emptying can be slowed by stress, meal size and lack of dietary moisture. Fasting for 12-24h before any procedure was extrapolated from human medicine and is now being questioned. Recent studies in dogs suggest shorter fasting times of 3-4h can be beneficial, whilst fasting for longer than 10h can increase the acidity in the stomach. Currently a fasting time of 6-8h is considered sufficient in domestic cats. Jaguars, on thein contrast to domestic cats, are often fed larger meals at greater intervals. It would therefore be advisable to provide a small meal on the evening before a planned procedure. Water should be available to the animal until the time of immobilisation.

Similar to other felids, anaesthesia of jaguars is usually uneventful if proper drugs and techniques are used. Drug dosages should be based on ideal bodyweight. However, the clinician should always be prepared for handling emergency situations (West *et al*, 2014) as these can and do arise.

2.8.7.2 Induction

There are several anaesthetic protocols that have been successfully used for free-ranging and managed jaguars. A review of these protocols can be found in Deem and Karesh, 2005; Deem, 2002;

and West *et al*, 2007. An important point is that clinicians should be familiar with the drugs they choose to use and understand the risk and benefits associated with different anaesthetic options. Following are three protocols that have been used in jaguars. All are administered intramuscularly (IM). Any adverse reactions should be reported to the EEP veterinary advisor.

- **Tiletamine/zolazepam** (e.g., Zoletil[®], virbac) 4–8mg/kg, IM. Unlike tigers (Armstrong, 1990), there are no reports of adverse reactions to Zoletil[®] in jaguars. As with other felids it's use is contraindicated in pregnant animals, those with pancreatitis, renal disease, or urethral obstruction (Clarke *et al*, 2014).
- Ketamine 3.5-4 mg/kg and xylazine 1-2 mg/kg, IM.
- Ketamine 2.5–4 mg/kg and medetomidine 0.05–0.07mg/kg, IM.

If excessive salivation is problematic, atropine (0.04 mg/kg) or glycopyrrolate (0.01–0.02 mg/kg) could be administered as a single dose either subcutaneously or intramuscularly. This should not be done lightly as in many species' atropine reduces salivation and airway secretions, but increases the viscosity, which can increase the risk of airway obstruction. Also, alpha2-agonists (medetomidine, xylazine...) cause peripheric vasoconstriction, therefore hypertension and bradycardia. Administration of atropine will increase the heart rate despite the high blood pressure, causing an even more pronounced hypertension. The heart will also have to pump at a higher frequency againstan increased resistance, increasing myocardial oxygen consumption, and if the animal has any previous electrolyte imbalance or cardiac disease there's an increased risk for arrythmias (Lerche, 2015).

Flumazenil is a specific benzodiazepine antagonist and can be used at a dose of 1/40th of the dose of tiletamine/zolazepam (0.1-0.2mg/kg). Atipamezole can be administered as a reversal agent at 1/5th of the xylazine dosage (0.2-0.4mg/kg) or 2 times the medetomidine dosage (0.1-0.14mg/kg) (Cordosa *et al*, 2011). In all the above a minimum of 30 minutes should be in between the initial injection and the administration of the reversal agent.

The absence of the 'ear flick reflex' can be used to check if the jaguar is sufficiently immobilized. This reflex is linked to the input of stimuli to the dorsal cochlear nucleus and when no flicking is observed it means that the animal is completely irresponsive to external stimuli which could arouse the cat (Bharathidasan *et al*, 2014).

2.8.7.3 Maintenance

The above protocols can be sufficient for quick procedures such as blood draws, placement of contraceptive implants... however if anaesthesia is to be maintained for longer (>30min) supplementary drugs AND oxygen will be required. Tiletamine/zolazepam, medetomidine or xylazine should be avoided as supplemental drugs. Aside from oxygen it is also advised to provide intravenous fluids at a base rate (10ml/kg/h), this supports the patient, but also provides direct intravenous access in case of emergency.

2.8.7.4 Injectable anaesthesia

Supplemental anaesthesia can be achieved by administering ketamine at a dose of 1–1.5 mg/kg IV, or 1–2 mg/kg IM as needed to maintain an adequate level of anaesthesia. Ketamine is cleared in part unchanged by the kidneys, caution is therefore needed when renal function is impaired. Propofol at a dose of 2mg/kg IV can be used to prolong anaesthesia for about 10-15min or it can be used in a continuous rate infusion (CRI) at 0.1mg/kg/min (Bharathidasan *et al*, 2014; Selmi et al, 2005). A dose dependent cardiorespiratory depression is a known side-effect. Felids are also deficient in glucuronosyltransferase, causing prolonged recoveries with repeated propofol boluses or CRI. It is advised to adjust the dose downward over time. Daily repetitive use in cats can cause Heinz body formation, delayed recovery, anorexia, diarrhoea, and malaise (Court, 2013). When repeated anaesthetics are required propofol should not be the drug of choice.

Alfaxalone, now often used in domestic cat medicine, might be an alternative for alpha2-agonists (hyperkalaemia) or propofol (repeated dosing). It gives dose-dependent, rapid and reliable sedation when administered IM, SC or IV. Reported alfaxalone dosage range for sedation in cats is 2-5mg/kg, after premedication the lower end of the dose range should be used (Rodrigo-Mocholí *et al*, 2018). In combination with an opioid or benzodiazepine 0.5-2mg/kg is advised in cats. In the literature only one case report in a wild felid, *Panthera pardus*, can be found. They used an IM combination of alfaxalone (1.6mg/kg) and ketamine (3.1mg/kg) as induction followed by inhalation anaesthesia. In the first 10 minutes 2 further intravenous injections of alfaxalone (1.6mg/kg each) were given to increase anaesthetic depth (Jimenez *et al*, 2020). At time of writing no other reports are available of its use in wild felids. Downside to this drug is the fact that required volumes are larger which limits its usefulness if darting is required.

2.8.7.5 Inhalation anaesthesia

Inhalation anaesthesia should be used wherever possible. It allows for a more controlled anaesthetic plane and a smoother recovery. Halothane, isoflurane, and sevoflurane can all be used. Isoflurane is however the most common anaesthetic gas in veterinary medicine. The isoflurane/oxygen mixture is administered through an endotracheal tube (internal diameter 11mm often suitable) which is placed after application of a local anaesthetic spray to the larynx. This prevents damage through laryngeal spasms. The ET-tube is placed correctly if its tip is level with the point of the shoulder, this correlates with the thoracic inlet.

Considering the weight of jaguars, a rebreathing system (circle anaesthetic system) should be used. This reduces the anaesthetic gas requirement by extracting the CO² out of the exhaled air and recirculating the oxygen and anaesthetic gas. The system needs to be saturated at the beginning of the anaesthetic. To achieve this oxygen is supplied at 2l/min and isoflurane at maximum 3% for the first 5 minutes. This allows the anaesthetic gas to be distributed evenly in the system/patient. Following this oxygen flow rate should be adapted for each individual animal. In a circle system, which has a circuit factor of 1, this equals the tidal volume (TV) as respiratory rate does not need to be considered. Tidal volume should always be based on the ideal weight of the individual as change in body score does not change the lung volume!

Oxygen flow rate (I/min) = tidal volume (10ml/kg)

In practise, oxygen flow is most often placed at a higher rate 1-2L/min. This way the system is not fully, but partially rebreathing. However, it compensates for minor leaks; isoflurane can still be set ata low % and the CO² absorbent (soda lime) lasts longer. Modern vaporizers are accurate with a flow as low as 250ml/min and they can more safely be used during low flow anaesthesia (Mosley, 2015).

For isoflurane the minimum alveolar concentration (MAC) that prevents movement in 50% (MAC⁵⁰) of felids exposed to a surgical incision is 1.6%. Maintenance levels of isoflurane are therefore best kept between MAC⁵⁰ and MAC⁹⁵ or 1.6 - 2.4%. But MAC is influenced by several factors. Increasing age and the use of premedication decreases MAC. In the case of *Panthera onca* we give fullanaesthetic dosages to be able to approach the animals. This needs to be considered when starting inhalation anaesthesia. Often only oxygen is supplied in the beginning and isoflurane added in only when signs of a lightening plane of anaesthesia are observed (Clarke *et al*, 2014). Keep in mind that when changing isoflurane vaporizer setting, the oxygen flow can be increased from maintenance for a few minutes to speed up the change both up and down more quickly.

The size of breathing bag required can be calculated as follows: **TV x 6**. This bag provides a compliant reservoir of gas and can, in absence of a mechanical ventilator, be used to supply intermittent positive pressure ventilation by just pushing on it. Amount of pressure should be adjusted by viewingthe chest expansion.

2.8.7.6 Monitoring

Anaesthetic monitoring is the key to a good anaesthetic. As a minimum heart rate, respiration and body temperature should be monitored. Following are the normal rates for jaguars:

- Body temperature: 37 39.5 °C
- Pulse: 70 140 beats per minute
- Respiration: 8 24 breaths per minute

It is advised to have more in-depth monitoring of vital signs definitely during longer procedures. This way problems can be detected and addressed in a timely manner. Available monitoring equipment includes:

- Oesophageal stethoscope
- Rectal thermometer
- Pulse oximeter
- ECG
- Capnograph
- Blood pressure monitor: cuff width should be 40% of the limb's circumference

2.8.7.7 Anaesthetic problems

No matter how well we anticipate and prepare problems can occur during any anaesthetic procedure. Some as treated by Robertson *et al*, 2018. are mentioned below. Again, this is by no means an exhaustive list.

Hypothermia

Hypothermia or low body temperature is most often caused by low environmental temperatures and/or a long surgical procedure. Hypothermia causes a decrease in cardiac output and thus blood pressure. Around 32°C asystole and fibrillation can occur. Increased blood viscosity and prolonged coagulation time cause an increase in bleeding. Poor perfusion leads to metabolic acidosis and decreased liver function plays a part in a prolonged recovery. Requirement for inhalant agent drops and an overdose becomes more likely. Peripheral vasoconstriction and impaired immune function can affect post-operative wound healing (Robertson, 2015). Monitoring the temperature is thus very important and when the temperature of a jaguar drops below 37°C supplemental heat will need to be provided. Intravenous fluids should be warmed, blankets, heat pads... can be placed on, under or around the animal.

Hyperthermia

On the other end of the spectrum, we have hyperthermia, that is just as detrimental to the animal. High environmental temperature, direct sunlight, pre-anaesthetic excitement or a drug reaction (opioids, tiletamine/zolazepam) can all play a role. Hyperthermia can lead to impaired enzyme function, proteindenaturation, coagulopathies, and cell death. Brain, kidneys, heart, and liver are the organs at greatest risk. During recovery hyperthermic patients can exhibit a dissociative state with restless movements and head bobbing. If the animal's temperature goes up to 40 °C its temperature must be brought back down. Wrapped ice packs can be placed in the inguinal area, cold water can be rubbed into the fur, alcohol can be placed on the foot pads, fans can be used to bring the temperature down (Clark *et al*, 2014). Fresh gas flow should be increased to wash out the warmed rebreathed gasses created by the reaction between CO^2 and soda lime. Intravenous fluids should be cooled.

Bradycardia

Bradycardia is defined as a heart rate reduction by 30% or more. If the animal received an alpha²agonists (xylazine, medetomidine) and has a normal blood pressure then atipamezole should be administered intramuscularly, however if the animal is hypotensive atropine can be administered intravenously at 0.01mg/kg. In all other cases the cardiac rhythm should be evaluated on the ECG.

With a normal rhythm the depth of anaesthesia and temperature of the animal needs to be double checked. Isoflurane level needs to be adjusted if the animal is too deep and heat needs to be added if the animal is hypothermic. If this doesn't help, the animal isn't hypothermic and if the anaesthetic depth is normal, then atropine at 0.01mg/kg can increase the heart rate.

With an abnormal rhythm (sinus arrest, 2nd degree AV block or synchronous AV dissociation) atropine can also help. In the case of a 3rd degree AV block ideally the procedure needs to be aborted. If this isn't possible dopamine or epinephrine can be administered to increase the heart rate. Small P waves and enhanced T waves are indicative of **hyperkalaemia**. This is a known complication in large felids and has been reported in jaguars (Romano *et al*, 2018). If left untreated cardiac arrest follows. In cases of hyperkalaemia a bolus of a short-acting insulin (0.5U/kg) should be administered IV, followed by an IV bolus of 25% dextrose at 2g/U insulin. This helps with the intracellular uptake of

potassium. Dextrose CRI can be continued afterwards. Cardiac conduction can be stabilised by calcium gluconate 10% (0.5-1.5ml/kg IV) given over 5-10 min whilst monitoring the ECG.

<u>Tachycardia</u>

All known normal heart rates in jaguars have been collected during anaesthetic procedures. Therefore, we don't have a normal resting heart rate to fall back on in this species. When referencing dogs of a similar weight tachycardia in jaguars could be defined as a heart rate above 140bpm. It is important to determine the baseline heart rate for your patient as soon as possible.

First, the anaesthetic plane needs to be evaluated. Tachycardia can be caused by both a light anaesthetic plane, all or not accompanied with pain stimuli, and a deep anaesthetic plane. In the first case the circuit should be checked for leaks (correct endotracheal placement...?) and the isoflurane level needs to be increased. If pain is suspected an analgesic should also be added. Often hyperventilation is present. Bagging the animal to give deeper breaths helps to increase the isoflurane uptake and speed up the deepening of the anaesthetic plane. When, on the other hand, the anaesthetic plane is to deep, hypotension can lead to tachycardia. The animal will have shallow slow breaths and a low end-tidal CO_2 (ETCO₂) on a capnograph due to hypoventilation, but on this occasion the isoflurane level needs to be decreased.

Presence of hypoxaemia, hypotension, hyperthermia needs to be evaluated and treated accordingly. Any of the following medications should be discontinued: atropine, dopamine, dobutamine, epinephrine, ketamine, ephedrine.

Hypotension

Hypotension in cats is defined as a mean arterial pressure (MAP) below 60mmHg and a systolic arterial pressure of less than 90mmHg. Do be aware that, because of compensatory vasoconstriction, MAP only drops after 15-20% acute total blood loss in an animal with a healthy heart.

To understand and correctly treat hypotension 3 factors must be considered: heart rate (HR), stroke volume (SV, amount of blood pumped at each beat) and systemic vascular resistance (SVR).

MAP = HR x SV x SVR

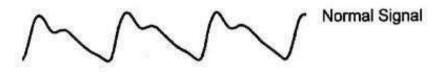
If **HR** goes up, MAP goes up (until a certain limit), when the HR goes down, MAP goes down.

To increase the **SV** you can attempt to increase the contractility by administering drugs such as dobutamine, dopamine and ephedrine. Doing this increases the actual volume the heart is pumping out and should increase MAP. Another way SV can be increased is by giving fluid boluses (crystalloid, colloid), which increases the venous return and allows the ventricles to get fully filled. This, however, will only work if the heart is fluid dependent.

SVR refers to the resistance to blood flow offered all the vasculature excluding the pulmonary system. Almost 70% of the total blood volume is stored in small veins. Increasing vasodilation (isoflurane for example) allows those vessels to hold more blood which reduces the venous return of the heart and thus the MAP. If the vascular tone increases (vasoconstriction) the opposite happens. Very marked vasoconstriction can oddly give a high MAP but with a decreased SV, this because of an increase of resistance to the blood flow out of the heart (increased after-load).

Keeping the above in mind, when hypotension is present, the anaesthetic depth is again the first point of interest and if the vapor setting can be reduced it should be (Clarke *et al*, 2014). If isofluranelevel reduction isn't improving the hypotension and the animal is also bradycardic than the latter problem needs to be treated. However, if the heart rate is normal the animal might be fluid dependant. This can be evaluated by looking at the pulse oximeter wave (plethysmogram, see figure below) presence of fluid loss or bleeding, and haematocrit and total protein levels. A crystalloid bolus (3-10ml/kg) should be administered over 10-15 minutes which can be repeated once. If no effect a colloid can be given slowly (1-5ml/kg).

Common pulsatile signals on a plethysmogram (Jubran, 1998)



Normal signal showing the sharp waveform with a clear dicrotic notch.



Pulsatile signal during low perfusion showing a typical sine wave.

Noise Artifact

Pulsatile signal with superimposed noise artifact giving a jagged appearance.

Motion Artifact

Pulsatile signal during motion artifact showing an erratic waveform.

If fluids don't help the animal is probably not fluid dependent. Then the increasing heartcontractility or heart rate should be attempted: dopamine infusion (0.05-0.1mg/kg/min),

dobutamine infusion (0.01-0.05mg/kg/min) or ephedrine 0.1-0.2mg/kg IV injection can be given. A cardiac work up might be worthwhile in this animal.

<u>Hypoxemia</u>

Hypoxemia is defined as a blood arterial haemoglobin saturation level below 95%. The typical blue discoloration of mucosae (cyanosis) can only be seen if the saturation drops below 67% or lower in anaemic patients. Hypoxaemia can be present when mucosae are still nice and pink. It is therefore best to base diagnosis of hypoxaemia on pulse oximeter readings. First thing is to check if the animal still has a pulse, if this is not present CPR needs to be started: chest compressions (100-120 compressions/min), bagging pure oxygen every 10s and administering epinephrine (0.01-0.02 mg/kg iv) (Romano et al 2018). If a heart rate is present a few manual breaths should be given and evaluated with a stethoscope to make certain air is moving through trachea and lungs (airway obstruction, mucus plug, pneumothorax...). If the respiratory system is functioning normally, you should check if the oxygen is getting to the patient, so every aspect of the circuit needs to be looked at. If oxygen supply and delivery is normal the SpO₂ probe should be evaluated to see it functions correctly and if needed moved to another location.

2.8.7.8 Ventilation

Ventilation is evaluated by looking at ETCO₂ and inspired CO₂. The capnograph is invaluable in evaluating potential problems (see Appendix 7). Awake physiological ETCO₂ values are between 35-45mmHG.

Hyperventilation = ETCO₂ less than 35mmHg. The circuit needs to be checked: leaks, correct intubation, disconnection. A rapid respiratory rate (ventilation of dead space) could be indicative of an inadequate anaesthetic plane. If IPPV manual or mechanical is used, you should maintain EtCO2 between 35 and 60 mmHg. The only exception is when increased intracranial pressure is suspected in which case the pressure should be kept around 32mmHg (Hammond and Murison, 2016).

Hypoventilation = ETCO₂ more than 60mmHg. Anaesthetic depth might be excessive, or the body position could impair breathing. Other causes are airway obstruction, bronchoconstriction, fluid or mass in the thorax, pressure on the thorax (surgeon?), increased abdominal pressure (laparoscopic procedure?) or inadequate IPPV.

If the inspired CO_2 is more than 5mmHg it indicates the animal is **rebreathing CO_2**. The soda-lime might be exhausted, or a valve might be malfunctioning.

The monitor itself can also malfunction. This is easily checked by disconnecting the machine. Room air should read 0 and when you breathe through it the value should be between 35-45mmHg.

2.8.7.9 Prolonged recovery

We unfortunately can't maintain intubation or monitoring as adequately as with domestic cats during the recovery process due to the nature of our patients. This limits our ability to interveneonce the jaguar is in recovery. Due to this constraint, it is very important to make certain hypoxemia, hypothermia... are corrected, and any possible antagonists administered before the animal is placed back in its enclosure. If possible, the animal should be positioned next to a fence to still allow oxygen supplementation or injection without entering the enclosure.

2.8.8 Post-mortem examination

A complete post-mortem examination should always be carried out whether the cause was clear at time of death or not. This is important because it gives information on the presence or absence of concurrent disease which might prove important for other individuals in the collection as well as the species. This is also the case for still-born animals or neonates.

Autolysis onset is quick, and decomposition will cause a lot of information to be lost. When detected the carcass should be maintained at 4°C and examined as soon as possible after death. Consistency is important as it allows comparative data to be collected and trends to be detected. It is therefore advised to follow a necropsy protocol (see appendix 8) and report all findings in a standardised matter. Ideally this report should be written in English and forwarded to the EEP coordinator to monitor for recurring health problems.

Samples for histopathology should be collected from all major organs as well as from any lesions that can be seen during gross post-mortem. Where indicated samples need to be taken for bacteriology, parasitology, virology.... Many institutions utilise private labs, partner with universities or have their own in-house pathology department to analyse these samples. It is also useful to consider storing tissues at -20°C or below. This may allow further diagnostic analyses later and may be useful for research purposes. The EEP Coordinator should be contacted for special requests regarding tissue and data collection, e.g., for urogenital tracts of chemically contracepted females.

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4 Appendices

Appendix 1: Example Diet Sheets (Chester Zoo)

Below diet sheet for jaguar 'Goshi', a female aged 6 years, at Chester Zoo.

Jaguar (*Panthera onca*)



Calf carcass, pelvis Calf carcass, front leg Rabbit, whole Chicken, whole Calf carcass FEEDING SCHEDULE Carnivore supplement – Mazuri Calf carcass, pelvis Calf carcass, front leg Rabbit, whole Chicken, whole	M X X	~(1 (~ 200 g (~2	ANTITY 5.5 g 1 1 1.5 kg) 1.5 kg) 2 small fish) 0.5 Au W X	ny changes to	these quanti		Y QUANTITY	
Carnivore supplement – Mazuri Calf carcass, pelvis Calf carcass, front leg Rabbit, whole Chicken, whole Chicken, whole Calf carcass	X	~4 1 (~ 200 g (~2	5.5 g 1 1 1.5 kg) 1.5 kg) 2 small fish) 0.5 Au		these quanti			
Carnivore supplement - Mazuri Calf carcass, pelvis Calf carcass, front leg Rabbit, whole Chicken, whole Coard or trout Calf carcass Carnivore supplement - Mazuri Calf carcass, pelvis Calf carcass, pelvis Calf carcass, front leg Rabbit, whole Chicken, whole Chicken, whole	X	~4 1 (~ 200 g (~2	5.5 g 1 1 1.5 kg) 1.5 kg) 2 small fish) 0.5 Au		these quanti			
Calf carcass, pelvis Calf carcass, front leg Rabbit, whole Chicken, whole Calf carcass FEEDING SCHEDULE Carnivore supplement – Mazuri Calf carcass, pelvis Calf carcass, front leg Rabbit, whole Chicken, whole	X	1 (~ 1 (~ 200 g (~2	1 1 1.5 kg) 2 small fish) 0.5 Au W			ties must be	noted on the	daily con
Calf carcass, front leg Rabbit, whole Chicken, whole Calf carcass Calf carcass Carrivore supplement – Mazuri Calf carcass, pelvis Calf carcass, front leg Rabbit, whole Chicken, whole	X	1 (~ 200 g (~2	1 1.5 kg) 2 small fish) 0.5 Au			ities must be	noted on the	daily con
Rabbit, whole Chicken, whole Chicken, whole Roach or trout Calf carcass Calf carcass Carnivore supplement – Mazuri Calf carcass, pelvis Calf carcass, front leg Rabbit, whole Chicken, whole	X	1 (~ 200 g (~2	1.5 kg) 1.5 kg) 2 small fish) 0.5 Au			ties must be	noted on the	dailu ran
Roach or trout Calf carcass	X	200 g (~2	2 small fish) 0.5 Au			ties must be	noted on the	dailu ran
Calf carcass	X	Т	0.5 Ai			ties must be	noted on the	daily con
ESEDING SCHEDULE Carnivore supplement – Mazuri Calf carcass, pelvis Calf carcass, front leg Rabbit, whole Chicken, whole	X	T	A/			ties must be	noted on the	dailu ron
Carnivore supplement – Mazuri Calf carcass, pelvis Calf carcass, front leg Rabbit, whole Chicken, whole	X	-	w			ties must be	noted on the	daily ron
Carnivore supplement – Mazuri Calf carcass, pelvis Calf carcass, front leg Rabbit, whole Chicken, whole	X	-		T				uuny rep
Carnivore supplement – Mazuri Calf carcass, pelvis Calf carcass, front leg Rabbit, whole Chicken, whole	X	-		T				
Calf carcass, pelvis Calf carcass, front leg Rabbit, whole Chicken, whole	X	-			F	S	5	Othe
Calf carcass, pelvis Calf carcass, front leg Rabbit, whole Chicken, whole				х	r X	X	X	othe
Calf carcass, front leg Rabbit, whole Chicken, whole	~			^	^	~	^	
Rabbit, whole Chicken, whole			x					
		x						
Roach or trout							x	
				x				
Calf carcass					X			
PREPARATION NOTES				TION NOTES				
Prey should be gutted if it has been previously froz • Beef is cut to required size • Supplement is sprinkled onto the chicken, rabbit a supplement is given with the fish		f but no	 Prey can b Food may branches, h Food may Ropes mag 	e fed whole y be hidden a nollow bamb y be floated o y be attache d manoeuvr	for enrichme round enclo oo, or cardb on rafts in po ed to prey to	sure, or in s oard boxes ol drag it arou		

Team:	
Carnivores	

Below diet sheet for jaguar 'Napo', a male aged 7 years, at Chester Zoo.

Jaguar (*Panthera onca*)



DIET SHEET FOR								
Napo								
DIET INGREDIENTS								
	QUANTITY					TEMPORAR		,
Carnivore supplement – Mazuri			.5 g					
Calf carcass, back leg			1					
Calf carcass, front leg			1					
Rabbit, whole		1 (~	2 kg)					
Chicken, whole		1 (~	2 kg)					
Roach or trout		200 g (~2	small fish)					
Calf carcass		0	.5					
	•		AI	ny changes to	these quant	ities must be	noted on the	daily repor
FEEDING SCHEDULE								
	м	Т	W	Т	F	S	5	Other
Carnivore supplement – Mazuri	X	X	X	x	X	X	X	
Calf carcass, back leg	X							
Calf carcass, front leg			X					
Rabbit, whole		X						
Chicken, whole							X	
Roach or trout				x				
Calf carcass					X			
PREPARATION NOTES			PRESENTA					
 Prey should be gutted if it has been previously 	frozen		•Prey can be fed whole for enrichment					
 Beef is cut to required size 			Food may be hidden around enclosure, or in sacks suspended from					
 Supplement is sprinkled onto the chicken, rab 	bit and beet	f but no	branches, hollow bamboo, or cardboard boxes					
supplement is given with the fish			 Food may be floated on rafts in pool 					
			Ropes may be attached to prey to drag it around the enclosure or					
			suspend and manoeuvre it with pulleys					
ATUER MOTE								
OTHER NOTES *1 level scoop of 5 mL white plastic scoop								
Tievel scoop of 5 mc white plastic scoop								

Team: Carnivores Diet version: 4.1

Appendix 2: Body Condition Scoring Chart (AZA)

(1) Extreme Low: emaciated, no fat covering, skeleton sharply visible	 Forequarters: Neck thin & deeply shrunken.Shoulder wasted, vertical line of scapula visible. Angles of shoulder blade & arm sharp. Bones & joints clearly visible. Midsection: Multiple ribs visible with deep depressions between. Individual vertebrae visible. Waist shrunken & tucked. Sharp angles dividing shoulder, torso & hip.
	Hindquarters: Point of hip & ischium sharp & clearly visible. Bones of leg (femur & knee) clearly visible. Flank sunken. Sacral vertebrae visible. Tail base protruding above hip.
(2-3) Low: minimal fat covering, articulations angular & some bones visible	Forequarters: Neck thin & shrunken, sinews apparent but flowing into shoulder. Angles & bones of shoulder & arm prominent but with slight covering. Peak of scapula prominent.
	Midsection: Multiple ribs visible; abdominal muscles may be apparent. Vertebrae may be visible. Waist/belly shrunken & tucked. Abdominal skin flap may be apparent but not filled. Clear definition between shoulder, torso & hip.
	Hindquarters: Point of hip prominent but slightly covered. Ischium visible but blunt. Muscle & bones of upper leg angular but softened by slight covering. Sacrum flat or slightly depressed. Tail base becoming visible.
(4) Moderate-Low: slight fat covering, bones barely noticeable, articulations apparent but smooth	Forequarters: Neck cylindrical but discernible from shoulder. Muscles of shoulder apparent but slightly smooth. Peak of scapula apparent.
	Midsection: Some ribs visible; abdominal muscles apparent. Vertebrae rarely visible. Waist evident. Abdominal skin flap may be apparent with nominal filling. Smooth but noticeable delineation between shoulder, torso & hip.
	Hindquarters: Point of hip visible but covered. Ischium noticeable but rounded. Muscles of upper leg apparent & smoothed by slight fat covering. Sacrum sloped, but beginning to fill & round.

(5) Moderate: slight fat covering, bones not apparent, articulations visible but smooth	 Forequarters: Neck filled in to flow smoothly into shoulder. Muscles of shoulder noticeable but with smooth covering. Peak of scapula noticeable but topline flows smoothly from neck into torso. Midsection: Ribs not visible; abdominal muscles noticeable as a transverse line. Vertebrae notvisible; back smooth or heart-shaped & muscled. Waist noticeable as a smooth concavity. Abdominal skinflap may be present with nominal filling. Slight delineation between shoulder, torso & hip. Hindquarters: Point of hip barely noticeable, well-rounded. Ischium noticeable when walking, barely noticeable (flat/straight) when standing. Upper leg smooth & filled with slight muscle definition. Slope of hip becoming rounder
(6) Moderate-High: noticeable fat covering, articulations becoming less noticeable	 Forequarters: Neck filled & becoming continuous with shoulder. Muscles of shoulder apparent but covered with fat. Peak of scapula noticeable but topline flows smoothly from neck into torso. Midsection: Ribs not visible; abdominal muscles barely noticeable. Back smooth & rounded, becoming flat. Waist less discernible as a shallow depression. Abdominal skin flap filling with fat. Delineation between shoulder & torso but torso flows smoothly into hip. Hindquarters: Point of hip & ischium completely rounded. Upper leg smooth & rounded, muscle definition minimal/barely noticeable. Sacrum rounded.
(7-8) High: considerable fat covering, animal becoming rounded & bulging	 Forequarters: Cheeks beginning to fill. Neck continuous between head & shoulder. Muscles of shoulder barely noticeable, covered in fat. Fat accumulating under neck & chest, & behind shoulder & arm. Midsection: Abdomen smooth, rounded & well-covered; ribs or muscles not visible. Back rounded & becoming flat. Waist almost filled, barelydiscernible. Abdominal skin flap apparent & thickened with fat. Shoulder barely differentiatedfrom torso, but torso continuous with hip. Hindquarters: Point of hip & ischium covered & rounded. Hip & upper leg filled & convexthroughout. Muscles of leg barely noticeable. Sacrum rounded, tail base flush & tail beginning to thicken.

(9) Extreme High: animal bulging & completely covered in a heavy fat layer



Forequarters: Cheeks filled out. Neck bulging & convex, continuous with head & shoulder. Shoulder rounded & muscles barely discernable. Pendulous fat accumulated beneath neck, chest, arms & abdomen.

Midsection: Torso rounded & smooth, continuous with shoulder & hip. Back smooth & rounded or flat & table-like. Waist filled & not discernible. Thick, pendulous fat along undercarriage.

Hindquarters: Hip & upper leg smooth & rounded. Muscles of leg not apparent. Tail noticeably thickened.

Appendix 3: Example Enrichment Devices and Methods (Chester Zoo)

Feeding	Description	
F1	Hessian Sacks	0
F2	Log piles	
F3	Bushes	
F4	Paper sacks	
F5	Buried in the ground	Manipulation/Cognative
F6	Hidden at the end of a scent trail	type feeding enrichment
F7	Under piles of leaves/grass/substrate	Frequency: once every 2
F8	Cardboard box	weeks
F9	Cardboard tube and hessian half buried	
F10	Bamboo feeder	
F11	Papier mache	
F12	Around water features	
F13	Hollow Log	
F14	Scatter	8
F15	Pool	
F16	Fixed to structure	
F17	Float	Physical type feeding
F18	Feeding pole	enrichment
E19	Hanging from string	
F20	High up in tree/forks	Frequency; few times a week
F21	High up with extendable pole	
F22	Tug of war post	
F23	Swing ball	<u></u>
Sensory		
81	Dung sack	
82	Scent trails Le citrus fruit	
\$3	Branches / substrate from prev animal	
84	Logs soaked in blood/stock	
85	Spices / tea / stock	
S6	Herbs / scented leaves i.e eucalyptus	
87	Blood soaked straw / logs	
S8	Calf skin / camel fur	17 10 10 10 10 10 10 10 10 10 10 10 10 10
S 9	Essential oil	PLEASE NOTE
Manipulatio	n	which substrate or scents used
M1	Wrecking Ball	
M2	Hessian sack filled with substrate	
M3	Papier mache	
M4	Cardboard box/paper sack	
M5	Log wrapped in hessian sacking	
MG	Ice block (please note type)	
M7	Bone	
MB	Wooden Ball	
M9	Rain maker	

ENRICHMENT DEVICES & METHODS

Appendix 4: EAZA Biobank Protocol





EAZA Biobank Sampling Protocol

NOTE: - ALL samples should be collected and shipped in accordance with national legislation

- Gloves should always be worn when collecting samples to prevent contamination
 - Samples may be taken from live animals opportunistically during routine veterinary or other procedures, or from deceased animals prior to sending away for necropsy.

General sampling procedure

Whole blood: up to 5mL in plastic EDTA (or

PAXgene) blood collection tubes. Invert 5 times to mix.

OR

Tissue: up to 1 cubic cm in plastic tube (such as a 2mL screw-cap tube) or bag. From live animals, tissue types include skin, muscle or umbilical cord Freeze at -20 or -80 °C immediately or preserve in ethanol (70-90%). NOTE: Do not use formalin or methylated alcohol.

Serum/plasma: 1-10mL in plastic tube. Must be spun and separated.

NOTE: serum or plasma should only be provided if it is accompanied by a blood or tissue sample.

Other sample types: For other sample types not listed above, contact the Biobank Coordinator. If a specific sample type is requested for an EAZA

Biobank approved project, it will be accepted by the EAZA Biobank.



Post-mortem considerations

For deceased animals, it is strongly encouraged to collect a sample before the carcass is sent away for necropsy or otherwise disposed of.

- Tissue: Collect at least 1 cm2 of skin (ex: ear margin), or 1 cubic cm of tissue from the tongue (this will not interfere with a post-mortem exam, if sent away). For in-house post-mortem exams, any tissue (muscle, liver, spleen etc.) or blood sample will be accepted. A larger sample volume, where possible will be accepted.
- NOTE: If sent for necropsy, it is also encouraged to agree with the institution(s) performing this service that a sample may be saved for the EAZA Biobank and is to be returned to the zoo.

Freeze at -20 or -80 °C immediately or preserve in ethanol (70-90%).

Group/colony sample collection

For animals managed in a colony or group management style, samples are still valuable, and we encourage collection and submission of samples from a portion of the group (example: aim to collect samples from approximately 10% of the group. Prioritize animals with individual identifiers and known unrelated animals).

NOTE: For small animals, a whole carcass can be submitted.

Data-only sample share

If an institution prefers to only make sample data available to the EAZA Biobank, rather than share a physical sample, please contact the Biobank Coordinator and see our data-only share guidance.

Storage and Shipping

Labeling

Label the sample tube or bag with animal identifier (GAN/ local ID), species, tissue type, and date of sampling.

Storage

Store samples in a freezer (-20 to -80°C) until shipment is possible. If preferred, collect samples over a period for batch shipment.

Packaging

- 1. Primary package: sample tube/ bag
- 2. Secondary packaging:
- 3. Tertiary packaging:



plastic container/bag with absorbent material (enough to absorb sample content) Cardboard box – include 'UN3373' diamond logo (see left) and the text "Biological substance Category B" and "refrigerate upon arrival" on the outside of the packaging.

NOTE: Please enclose the following in your shipment:

- ZIMS specimen report, or list of samples, EAZA Biobank MTA (if not sent electronically), and contact details of the sender.
- An icepack or dry ice to keep the samples frozen. Icepacks are not needed for samples in ethanol.
- Please contact the Biobank Hub to coordinate shipping and avoid arrival on a weekend.

ZIMS Sample storage and record sharing

It is possible to share sample data electronically via ZIMS Sample Storage module.

Please create a sample record in the ZIMS Sample Storage module, select record(s) to share and share with the 'EAZA Biobank' institution.



CITES

Some sample from certain species may require CITES Permits. General rules according to CITES regulations:



Within the EU: there is no need for CITES export/ import permits (exemptions may apply).

Outside the EU: CITES export permits must be applied for at the national CITES office. Remember to apply for permits ahead of shipping samples.

CITES Exemption: exemption is possible for a registered scientific institution (article VII, §6) All four EAZA Biobank Hubs have the CITES exemption, your institution may be eligible to apply



Biobank Hub Addresses

Please send samples to the Biobank Hub relevant for your country Contact the Hub before shipping samples.

Edinburgh Hub	Shipping Co	untry: UK, Ireland, Qatar, UAE, Kuwait		
	Att:	EAZA Biobank Samples		
RZSS	c/o:	RZSS WildGenes Biobank		
NLOO	Address:	Royal Zoological Society of Scotland (RZSS)		
to a subscription of the		134 Corstorphine Road		
		Edinburgh EH126TS, UK		
	Email:	biobank@rzss.org.uk		
Berlin Hub	Shipping Co	untry: Germany, Austria, Croatia, Czech Republic, Hungary,		
	Poland, Russia, Slovakia, Slovenia, Switzerland, Ukraine			
MAREN				
Pro Tar and	Att:	Dr. Jörns Fickel		
LT KI KING TAL	Address:	Department of Evolutionary Genetics Leibniz		
Leibniz Institute for Zoo and Wildlife Research		Institute for Zoo and Wildlife Research (IZW)		
and writerine research		Alfred-Kowalke Strasse 17		
		10315 Berlin, Germany		
	Email:	fickel@izw-berlin.de		
Antwerp Hub	Shipping Co Israel, Italy,	untry: Belgium, Luxembourg, The Netherlands, France, Greece, Turkey		
2				
	Israel, Italy,	Turkey		
	Israel, Italy, Att:	Turkey Dr. Philippe Helsen		
2	Israel, Italy, Att:	Turkey Dr. Philippe Helsen Centre for Research and Conservation		
	Israel, Italy, Att:	Turkey Dr. Philippe Helsen Centre for Research and Conservation Royal Zoological Society of Antwerp (KMDA)		
	Israel, Italy, Att:	Turkey Dr. Philippe Helsen Centre for Research and Conservation Royal Zoological Society of Antwerp (KMDA) Koningin Astridplein20-26		
ANTWERF ZOO SOCIETY	Israel, Italy, Att: Address: Email:	Turkey Dr. Philippe Helsen Centre for Research and Conservation Royal Zoological Society of Antwerp (KMDA) Koningin Astridplein20-26 2018 Antwerp, Belgium Philippe.Helsen@kmda.org		
	Israel, Italy, Att: Address: Email: Shipping Con	Turkey Dr. Philippe Helsen Centre for Research and Conservation Royal Zoological Society of Antwerp (KMDA) Koningin Astridplein20-26 2018 Antwerp, Belgium		
ANTWERF ZOO SOCIETY	Israel, Italy, Att: Address: Email: Shipping Col Sweden, Por Att:	Turkey Dr. Philippe Helsen Centre for Research and Conservation Royal Zoological Society of Antwerp (KMDA) Koningin Astridplein20-26 2018 Antwerp, Belgium Philippe.Helsen@kmda.org untry: Denmark, Estonia, Finland, Latvia, Lithuania, Norway, rtugal, Spain Dr. Christina Hvilsom		
ANTWERF ZOO SOCIETT	Israel, Italy, Att: Address: Email: Shipping Con Sweden, Por	Turkey Dr. Philippe Helsen Centre for Research and Conservation Royal Zoological Society of Antwerp (KMDA) Koningin Astridplein20-26 2018 Antwerp, Belgium Philippe.Helsen@kmda.org untry: Denmark, Estonia, Finland, Latvia, Lithuania, Norway, rtugal, Spain Dr. Christina Hvilsom Copenhagen Zoo		
ANTWERF ZOO SOCIETT	Israel, Italy, Att: Address: Email: Shipping Col Sweden, Por Att:	Turkey Dr. Philippe Helsen Centre for Research and Conservation Royal Zoological Society of Antwerp (KMDA) Koningin Astridplein20-26 2018 Antwerp, Belgium Philippe.Helsen@kmda.org untry: Denmark, Estonia, Finland, Latvia, Lithuania, Norway, rtugal, Spain Dr. Christina Hvilsom Copenhagen Zoo Roskildevej 38		
Copenhagen Hub	Israel, Italy, Att: Address: Email: Shipping Col Sweden, Por Att:	Turkey Dr. Philippe Helsen Centre for Research and Conservation Royal Zoological Society of Antwerp (KMDA) Koningin Astridplein20-26 2018 Antwerp, Belgium Philippe.Helsen@kmda.org untry: Denmark, Estonia, Finland, Latvia, Lithuania, Norway, rtugal, Spain Dr. Christina Hvilsom Copenhagen Zoo		

Samples may be donated or loaned to the Biobank, please see our Material Transfer Agreement. It is the responsibility of the lending institution to keep the Biobank up to date with contact details.

Appendix 5: Panthera onca CBC and Biochemistry Reference Ranges (Species360, 2021)

Biochemistry

			Reference	Sample	
Parameter	Unit	Mean	Range	Size	Animals
Sodium	mmol/L	150	137 - 162	52	23
Potassium	mmol/L	4	3.3 - 5.2	68	35
Chloride	mmol/L	120	112 - 128	620	201
total CO2	mmol/L	15.7	11 - 20.9	323	115
Calcium	mmol/L	2.4	2.1 - 2.9	716	237
Phosphorus	mmol/L	1.65	1.1 - 2.52	601	227
Magnesium	mmol/L	0.92	0.7 - 1.25	145	73
Glucose	mmol/L	8.08	4 - 14.8	730	246
BUN	mmol/L	9.4	5 - 16.8	733	249
Creatinine	µmol/L	160	54 - 265	736	246
BUN/crea ratio	ratio	15.1	6.4 - 33.7	205	100
ALT	U/L	68	25 - 150	692	235
AST	U/L	34	14 - 79	638	221
LDH	U/L	161	27 - 475	175	91
ALP	U/L	19	4 - 67	646	227
GGT	U/L	2	0 - 7	279	141
Amylase	U/L	1368	261 - 3276	287	141
Lipase	U/L	17	0 - 81	133	76
СК	U/L	265	79 - 813	433	178
Total bilirubin	µmol/L	3	0.2 - 6.8	644	216
Direct bilirubin	µmol/L	1	0 - 6.8	108	63
ТР	g/L	74	57 - 90	690	229
Globuline	g/L	41	22 - 70	561	203
Albumin/Globuline ratio	ratio	0.9	0.2 - 1.7	234	117
Fe	µmol/L	13.3	3.4 - 22.7	53	29
Bicarbonate	mmol/L	18.2	12.7 - 26	57	18
Cholesterol	mmol/L	6.27	3.39 - 9.69	562	216
triglyceride	mmol/L	0.3	0.13 - 0.74	247	118
рН	рН	6.4	5.0 - 8.0	122	61
Total thyroxine	nmol/L	17.5	0.9 - 42.7	47	37

CBC (Haematology)

			Reference	Sample	
Parameter	Unit	Mean	Range	Size	Animals
WBC	10^9/L	10.5	5.3 - 19.2	848	226
RBC	10^9/L	7.34	4.96 - 9.82	663	228
Hb	g/L	117	73-158	849	264
НСТ	%	36	23 - 49.5	289	125
MCV	fl	49	41.9 - 56.9	220	102
МСН	pg/cell	0.98	0.82-1.10	153	80
MCHC	g/L	325	276 - 370	204	100
Platelet count	10^12/L	0.273	0.101 - 0.520	565	190
Segmented neutrophils	10^9/L	7.94	0.01 - 16.13	77	40
Band neutrophils	10^9/L	0.35	0 - 2.28	51	27
Lymphocytes	10^9/L	1.6	0.14 - 4.81	131	65
Monocytes	10^9/L	0.3	0 - 0.964	130	62
Eosinophils	10^9/L	0.209	0 - 0.9	116	57
Basophils	10^9/L	0.004	0 - 0.034	104	46
Segmented neutrophils	%	76.7	39.8 - 94.4	11	56
Band neutrophils	%	5.4	0 - 85.5	58	34
Lymphocytes	%	18.6	3.8 - 56	133	67
Monocytes	%	2.8	0 - 8.6	134	68
Eosinophils	%	2	0 - 7.2	116	61
Basophils	%	0	0 - 0.3	106	51

Appendix 6: Radiographic Protocol

Both axial and appendicular skeleton are being evaluated.

Vertebral column radiographic changes can be assessed from images taken for thoracic and abdominal radiography if necessary. However, a view columnated to axial skeleton is ideal forassessing subtle changes of the intervertebral disc space.

Axial skeleton to be divided into:

- Cervical (C1-C7/T1 junction)
- Thoracic (T1-T13)
- Thoracolumbar junction
- Lumbar (L1-L7)
- Lumbosacral junction (L7-S1)

Lateral views are sufficient for all segments other than the lumbosacral junction. Extended ventrodorsal view of the pelvis is also required for lumbosacral junction, sacroiliac joint and bilateral coxofemoral assessment. To best assess caudal cervical spine, recommendation is that forelimbs be drawn caudad, to increase likelihood of pathology detection.

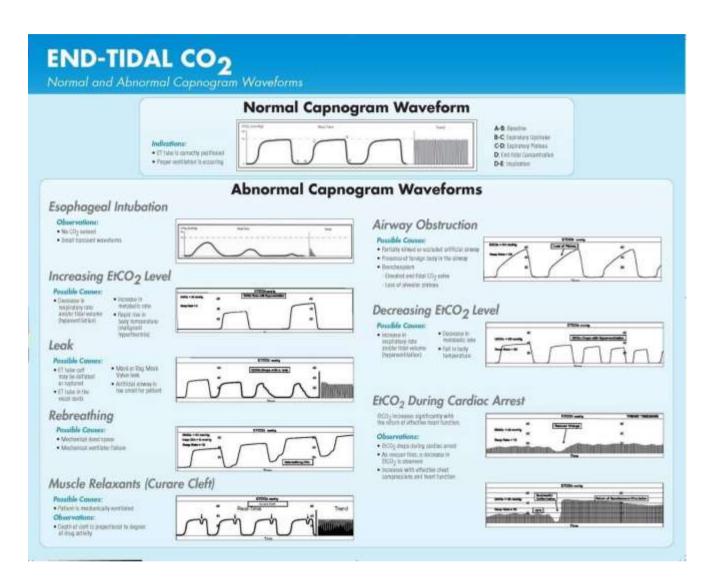
Appendicular skeleton in order of priority:

- Coxofemoral joints: extended ventrodorsal view, as above
- Elbow
 - mediolateral neutral angulation
 - o mediolateral flexed
 - craniocaudal projection if possible
- Stifle: mediolateral view should be sufficient. Although orthogonal projections are preferred, to date little additional information has been gained from attempted caudocranial or craniocaudal projections due to obliquity in positioning
- Tarsus
 - o mediolateral
 - o dorsoplantar
- Shoulder: lateral projection only, with joint overlying trachea, is usually sufficient for DJD detection
- Carpus
 - o mediolateral
 - o dorsopalmar

The above are guidelines only. Orthogonal views, though preferred, may not always be possible, and some requested projections may not be feasible. We would like to stress that all radiographs taken will be greatly valued and appreciated.

Additionally, ideally radiographs are to be matched with findings from physical examination performed at the time of imaging. For appendicular joints of interest, notations of observations regarding joint distension/ effusion/ thickening, crepitus, range of movement and associated muscle atrophy would be appreciated. Equally, any spinal kyphosis, lordosis or scoliosis, and associated muscle atrophy to be recorded.

Appendix 7: Capnograph Wave Forms



Appendix 8: Panthera Onca Necropsy Protocol (2021)

Animal identifi	cation				
Name		Date of Birth		Microchip number	
Sex		Offspring?		GAN-number	
Owner/ Collect	tion:				
Death related i	nformation				
Date		Time		Cause	
Necropsy relat	ed information				<u> </u>
Date		Time		Degree of composition	
Total weight	kg	Location		Performed by	
History, Anamr (enclosure, gro		contraception, nu	utrition, vaccination	ons, deworming, clinical sign	s, medical imaging, other)

Gross Post Mortem Findings

General Condition	
Orifices	
Skin / Coat	
Skirly Cour	

Musculoskeletal System	
Abdominal cavity	
Thorax	
Cardiovascular System	
Respiratory system	
Digestive System	
0	
Urogenital System	
orogenitai system	

Lymphoreticular System	
, , , ,	
Fuede avia a Cratana	
Endocrine System	
Nervous System	
Sensory Organs	
Selisory Organs	
Preliminary Diagnosis	
r reminary Blaghosis	

Relevant laboratory results

CBC	
ebe	
Biochemistry	
Diochernisery	
Deves alter le sur	
Parasitology	
Virology	
Bacteriology	

Histopathological findings

Samples for histopathology should be collected from all major organs as well as from any lesions that can be seen during gross post-mortem. Representative sections of organs should be collected in 10% buffered formalin. Do not place to much tissue in one container, ideally tissues are no thicker than 1cm and the ratio of 1-part tissue to 10-parts formalin should not be surpassed.

Organ	Results	Stored?
Lung		
Trachea		
Heart		
Diaphragm		
Gastrointestinal tract		
Pancreas		
Liver		

Spleen	
Kidney (L&R)	
Rancy (Early	
Adrenal glands	
Urinary bladder, ureter, urethra	
, , ,	
Devene du stive tre st	
Reproductive tract	
Lymph nodes	
Skeletal muscle	
Bone	
Skin	

Brain	
Spinal cord	
Eye	
LýC	
Thymus	
Thyroid, parathyroid	
Preliminary diagnosis	

Conclusion: Diagnosis, Cause of Death