EAZA Best Practice Guidelines for Elephants

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publication. Because the technical information provided in the EAZA Best Practice Guidelines can easily be misread or misinterpreted unless properly analyzed, EAZA strongly recommends that users of this information consult with the editors in all matters related to data analysis and interpretation.

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

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SECTION 1: BIOLOGY AND FIELD DATA

BIOLOGY

1.1 TAXONOMY

Order	Proboscidea
Family	Elephantidae
Genera	*Loxodonta* (African elephant)
	Elephas (Asian elephant)
Species	*Loxodonta africana* (African bush elephant)
	Loxodonta cyclotis (African forest elephant)
	Elephas maximus (Asian elephant)

Table 1 Elephant taxonomy

Species and Subspecies

Recent genetic and phylogenetic research suggests the presence of two species of African elephant (savannah *Loxodonta africana* and forest *Loxodonta cyclotis*), one species of Asian elephant (*Elephas maximus*) and a number of subspecies of both African and Asian elephants (Table 1). It is likely that elephant taxonomy may diversify in the future (Choudhury *et al*., 2008; Meyer *et al.*, 2017).

There are currently three or four recognized living subspecies of Asian elephants; the Sri Lankan subspecies (*E. m. maximus*), the mainland Indian subspecies (*E. m. indicus*), the Sumatran subspecies (*E.m. sumatranus*) and (according to some authors) the Borneo subspecies (*E.m. borneensis*). The Indian elephant (*E. m. indicus*) has the broadest distribution, extending between the southern and south-eastern regions of Asia.

Phylogeny

The order Proboscidea contains one living family, Elephantidae, the elephants, and several extinct families. Elephants are members of the broad evolutionary line leading to ungulates or hoofed mammals. Early proto ungulates showed extensive diversification in the Eocene. These fossils show development towards the ungulate condition, but the limbs remained primitive and the nails had not evolved into proper hooves. These lines died out leaving only the remnants of three: the sirenians (sea cows), hyraxes and elephants (Todd, 2010).

1.2 MORPHOLOGY

SPECIES	WEIGHT (kg)	HEIGHT (at shoulder, cm)	LENGTH (head and body, cm)
L. africana			
Male	4,000-6,000	280-400	500-650
Female	2,400-3,500	240-300	450-600
L. cyclotis			
Male	2,800-3,200	240-280	400-450
Female	1,800-2,500	210-240	350-400
E. maximus			
Male	5,400	240-300	550-640
Female	2,720	210-240	

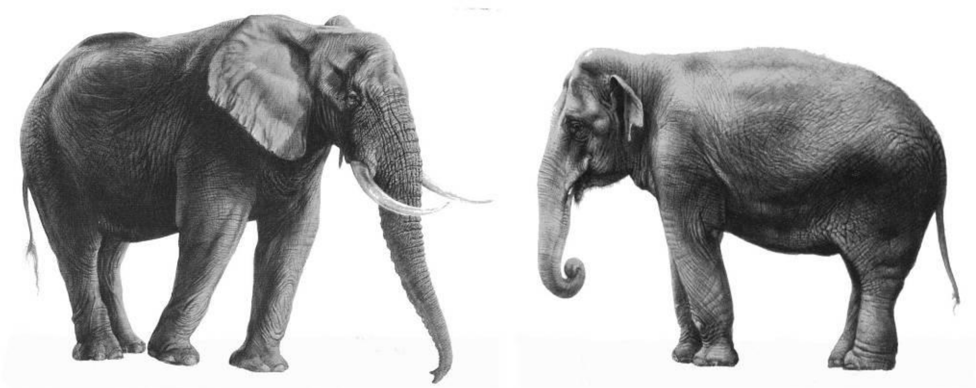
Table 2 Morphological data of elephants (Eltringham, 1982)

Elephants have a very large head and ears (especially in *Loxodonta*); the neck is short; the body and limbs are long, and the tail is of moderate length. Ears are used in communication and are also important in regulating body temperature. The feet are short and broad and columnar in shape. The weight rests on a pad of elastic tissue which acts as a cushion or shock absorber; there are five toes on each foot. The forefoot is circular in outline and the hind more elongated. The skin is sparsely haired and the sebaceous glands, which are associated with the hair follicles in most mammals, are not present in elephants thus there is no natural method for softening and lubricating the skin. Females have two nipples just behind the front legs and the testes are retained permanently within the abdominal cavity of males.

The second maxillary incisors grow to form the tusks, continuing to grow throughout life, one third being embedded in the skull. The extension of the pulp cavity has been shown to change with age and probably further factors (Steenkamp, 2008). The molar or grinding teeth are unique. The total number is 24; six in each half jaw but no more than two teeth are in use at any one time. There is linear progression with each tooth appearing at the back of the jaw and moving forwards as the preceding tooth is progressively worn down at the front (Kingdon, 1997). Each tooth drops out piecemeal as it reaches the front of the jaw (Eltringham, 1982).

The trunk is an information receptor; the elephant uses it as an olfactory organ, picking up scents and smells, and also to explore food and other items. It combines an elongated nose and the upper lip; the nostrils being located at the tip. The finger-like extremities are used to pick up objects which can be seized and manipulated with extreme sensitivity. The trunk can be used both for gentle caresses and admonitory slaps to the young. Charging elephants curl up their trunk to slap it out forwards energetically after folding its trunk back, using the forehead as a battering ram, its forefeet to kick or trample and the tusks to stab. According to Benedict (1936), the trunk can hold up to 4-8 litres, and perhaps >10 litres in an adult Asian male (Spinage, 1994). The trunk is essential to the survival of the elephant. Smell is the most highly developed sense; eyesight is limited but hearing acute. Midway between the elephant's eye and ear is a slit-like orifice, the temporal gland, which lies just beneath the skin. These glands emit a dark, strong-smelling oily substance, especially from bulls when in musth. Viscosity as well as olfactory quality of the temporal secretion varies with age, sex and reproductive status.

The differences between African and Asian elephants are well described (Carrington, 1962; Eltringham, 1982; Nowak, 1991; Shoshani, 1991). The main differences (see Fig. 1 and Table 2) are: the Asian is smaller, has a convex back and much smaller ears. It also has twin mounds on the forehead, whereas the African has a single dome. The trunk has two lips or 'fingers' in the African and one in the Asian. There are also differences in the surfaces of the molar teeth. Tusks are very short or even absent in female Asian elephants (termed 'tushes'). Occasional male Asian elephants do not grow tusks. All details on elephant foot differences and foot structure can be found in Csuti *et al.* (2001) or see chapter **2.8.1 Foot care**. Both genera have 56 chromosomes (Hungerford *et al.*, 1966).



African Elephant Asian Elephant

Figure 1 African and Asian elephant profiles

1.3 PHYSIOLOGY

As every species does, elephants express a variety of physiological traits accurately adapted to the environment they evolved in. We focus here on physiological characteristics specific to elephants and of practical relevance for their care.

In elephants, a heart rate of 25-50 beats per minute (bpm) is considered normal. It makes a big difference whether the elephant is in a standing (25-35 bpm) or recumbent (30-50 bpm) position. Normal parameters for the respiratory rate are 10-12 breaths per minute in a standing elephant, but can decrease to 4 breaths per minute in a sleeping animal (Wiedner, 2015).

For the African as well as the Asian elephant hematology reference values are available (Table 3 and 4). When analyzing hematological parameters of an individual elephant, the substantial inter- as well as intra-individual variation should be kept in mind. Based on that, regular monitoring and building up of an individual baseline will provide much more reliable benchmarks in the case of a disease (Perrin *et al.*, 2018).

Parameter	***Loxodonta***	***Elephas***
White blood cells (x10^9 cells/l = x10^3 cells/µl)	9.83 (9.56)	12.29 (11.81)
Red blood cells (x10^{12} cells/l = x10^6 cells/µl)	2.93 (2.90)	2.91 (2.86)
Hemoglobin (mmol/l) *(g/dl)*	7.94 (7.94) *12.8 (12.8)*	7.51 (7.45) *12.1 (12.0)*
Hematocrit (%)	37.0 (36.9)	35.6 (35.0)
MCV (fL)	125.5 (125.6)	122.3 (122.8)
MCH (fmol) *(pg)*	2.7368 (2.7368) *44.1 (44.1)*	2.5878 (2.6064) *41.7 (42.0)*
MCHC mmol/l *(g/dl)*	21.8 (21.7) *35.1 (35.0)*	21.2 (21.3) *34.1 (34.4)*
Segmented neutrophils (heterophils) (x10^9 cells/l *= x10^3 cells/µl)*	3.34 (3.03)	3.6 (3.09)
Lymphocytes (x10^9 cells/l *= x10^3 cells/µl)*	4.14 (3.81)	3.53 (2.74)
Monocytes (x10^9 cells/l *= x10^3 cells/µl)*	2.05 (1.56)	4.78 (5.29)
Eosinophils (x10^9 cells/l *= x10^3 cells/µl)*	0.175 (0.130)	0.293 (0.233)
Basophils (x10^9 cells/l *= x10^3 cells/µl)*	0.135 (0.105)	0.121 (0.115)
Platelets(x10^9 cells/l *= x10^3/µl)*	381 (313)	447 (411)

Table 3 Complete blood count values (International Units) given as mean (median) (Wiedner, 2015). Note that figures in Italics represent conventional units.

Parameter	***Loxodonta***	***Elephas***
Glucose (mmol/l) *(mg/dl)*	4.72 (4.72) *85 (85)*	4.83 (4.77) *87 (86)*
Blood urea nitrogen (mmol/l) *(mg/dl)*	3.21 (3.21) *9 (9)*	4.28 (4.28) *12 (12)*
Creatinine (µmol/l) *(mg/dl)*	115 (115) *1.3 (1.3)*	133 (133) *1.5 (1.5)*
Uric acid (µmol/l) *(mg/dl)*	11.9 (5.9) *0.2 (0.1)*	11.9 (11.9) *0.2 (0.2)*
Calcium (mmol/l) *(mg/dl)*	2.73 (2.70) *10.9 (10.8)*	2.65 (2.63) *10.6 (10.5)*
Phosphorus (mmol/l) *(mg/dl)*	1.58 (1.55) *4.9 (4.8)*	1.55 (1.52) *4.8 (4.7)*
Sodium (mmol/l = mEq/l)	129 (129)	131 (130)
Potassium (mmol/l = mEq/l)	4.8 (4.7)	4.6 (4.6)
Chloride (mmol/l = mEq/l)	89 (89)	91 (91)
Total protein (g/l) *(g/dl)*	78 (78) *7.8 (7.8)*	81 (82) *8.1 (8.2)*
Albumin (mmol/l) *(g/dl)*	0.466 (0.482) *3.1 (3.2)*	0.482 (0.482) *3.2 (3.2)*
Globulin (g/l) *(g/dl)*	46 (46) *4.6 (4.6)*	48 (48) *4.8 (4.8)*
Fibrinogen (g/l) (mg/dl)	2.64 (2.55) 264 (255)	3.89 (4.00) 389 (400)
Alkaline phosphatase (IU/l)	96 (88)	115 (101)
Lactate dehydrogenase (IU/l)	937 (984)	411 (336)
Aspartate aminotransferase (IU/l)	20 (18)	19 (18)
Alanine aminotransferase (IU/l)	8 (5)	9 (6)
Creatine kinase (IU/l)	223 (199)	177 (145)
Gamma-glutamyltransferase (IU/l)	10 (10)	6 (6)
Amylase (IU/l)	1798 (1214)	2247 (1008)
Lipase (IU/l)	9 (6)	17 (23)
Bilirubin, Total (µmol/l) *(mg/dl)*	3.42 (3.42) *0.2 (0.2)*	3.42 (3.42) *0.2 (0.2)*
Bilirubin, Direct (µmol/l) (mg/dl)	1.71 (1.71) 0.1 (0.1)	1.71 (1.71) 0.1 (0.1)
Bilirubin, Indirect (µmol/l) *(mg/dl)*	1.71 (1.71) *0.1 (0.1)*	1.71 (1.71) *0.1 (0.1)*
Cholesterol (mmol/l) *(mg/dl)*	1.87 (1.81) *72 (70)*	1.09 (1.09) *42 (42)*
Triglyceride (mmol/l) *(mg/dl)*	0.678 (0.60) *60 (53)*	0.57 (0.50) *50 (44)*
Bicarbonate (mmol/l = mEq/l)	25.6 (26.0)	24.9 (25.0)
Magnesium (mg/dl)	2.23 (2.30)	2.17 (2.14)
Iron (µmol/l) *(ug/dl)*	14.3 (14.3) *80 (80)*	11.3 (11.1) *63 (62)*
Carbon dioxide (mEq/l)	25.4 (25.5)	24.7 (25.0)
Progesterone (nmol/l) *(ng/ml)*	1.37 (0.95) *0.43 (0.30)*	1.08 (0.80) *0.34 (0.25)*
NB: the progesterone value much depends on the assay and analyzer used. Results from one type of analyzer cannot be compared with results from another type unless proper calibration has been performed.		

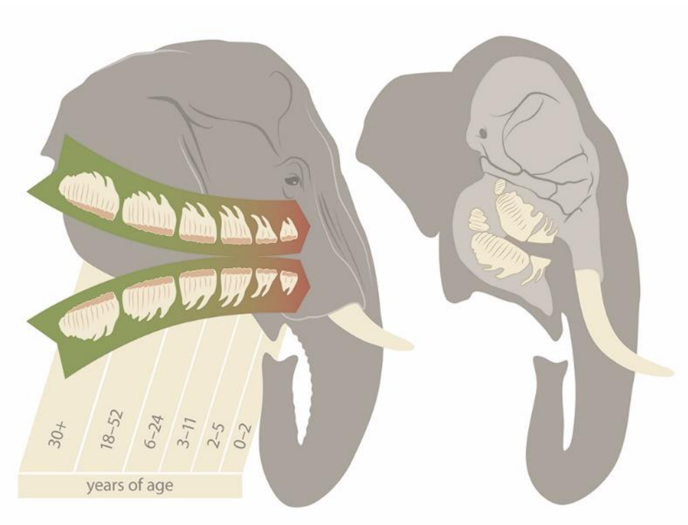
Table 4 Biochemistry blood values (International Units) given as mean (median) (Wiedner, 2015). Note that figures in Italics represent conventional units.

Naturally living in warm climatic regions and dealing with a very low body surface to body mass ratio, elephants need a sophisticated thermoregulation system. Although the individual variation is enormous, a baseline body temperature of 36-37°C is considered normal in an adult elephant (Wiedner, 2015). Body temperature is measured by placing the thermometer in a freshly passed fecal bolus. The center of the latter provides a more reliable value than rectal insertion would (Wiedner, 2015). Although measurement of oral temperature by the use of infrared thermometers is widely applied for monitoring purposes in zoos (e.g. EEHV monitoring in calves), defining the correlation of these values with body core temperature needs further research. In human medicine oral temperature shows a wide variance and weak correlation with body core temperature, and thus represents no recommendable approach for temperature measurement (Mazerolle *et al.*, 2011). Thermoregulation in elephants covers behavioral as well as physiological aspects. Behaviorally, elephants show bathing and mud bathing with subsequent sand dusting as well as resting in the shade to keep cool. To warm up they can be observed basking with the flank as the largest body part directed towards the sun. Physiologically, a diurnal pattern of body temperature has been reported in elephants (Kinahan *et al.*, 2007; Weissenböck *et al.*, 2012). This mechanism allows elephants to lower their core temperature during cold nights and increase it during hot days. Moreover, the elephant´s ear, with its highly developed net of superficial blood vessels, provides a comprehensive surface area for heat dissipation by convection. The latter´s efficacy can be increased based on demand by regular ear-flapping and demonstrates the synergy of physiological and behavioral mechanisms in thermoregulation.

Elephant skin in the hindquarter and caudo-lateral hind legs reaches a thickness of more than 3cm, however, there are body regions (e.g. medial part of the pinnae) with a skin thickness similar to that of humans (Shoshani, 1982). These variations should be kept in mind when planning blood sampling or parenteral application of drugs or evaluating the impact of climatic factors (e.g. frost bite on pinnae). Independent of its thickness, the elephant´s skin isa sensitive organ. Hence, the amount of natural behaviors related to skin care seems unsurprising. If elephants have the opportunity to do so, they may show extensive water and mud bathing, sand dusting, rubbing and scratching behavior of every body part. Besides skin care functions, these behaviors are part of thermoregulation, as mentioned above.

Apart from the continuously growing tusks (with a growth rate of around 18cm per year according to (Wiedner, 2015), elephants show a unique dental function called molar progression. In the course of the latter, molars show a stepwise horizontal shift towards the oral opening with the worn-down lamellae falling out piecewise (Fig. 2). This process results in varying size of the grinding surface which might impact chewing efficiency (Schiffmann *et al.*, 2019a). The dental formula of elephants is **I** 1/0 **C** 0/0 **m** 3/3 **M** 3/3 (I: permanent incisor, C: permanent canine, m: deciduous molar, M: permanent molar, maxillary/mandibular). In the African elephant, the presence of a deciduous tusk has been reported but this structure is reabsorbed without eruption and is considered to provide orientation for the permanent tusk (Raubenheimer *et al.*, 1995).

A) **B)**



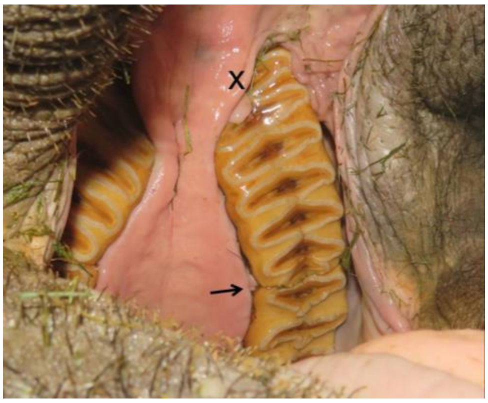


Figure 2 A) Schematic drawing of molar progression with indication of age stages in an African elephant (*Loxodonta africana*) and position of molars in the skull of an Asian elephant (*Elephas maximus*). *© UZH*
B) View of the oral cavity of an African elephant (*Loxodonta africana*). The arrow indicates the junction between two molars. Some lamellae of the more aboral one have already fallen out (x). © *Christian Schiffmann*

A further unique characteristic of elephants is the so-called pharyngeal pouch. This pouch is located ventrally in front of the epiglottis and allows the storage of some water (Fig. 3). Elephants may withdraw water from this pouch when it is scarce and use it to spray themselves

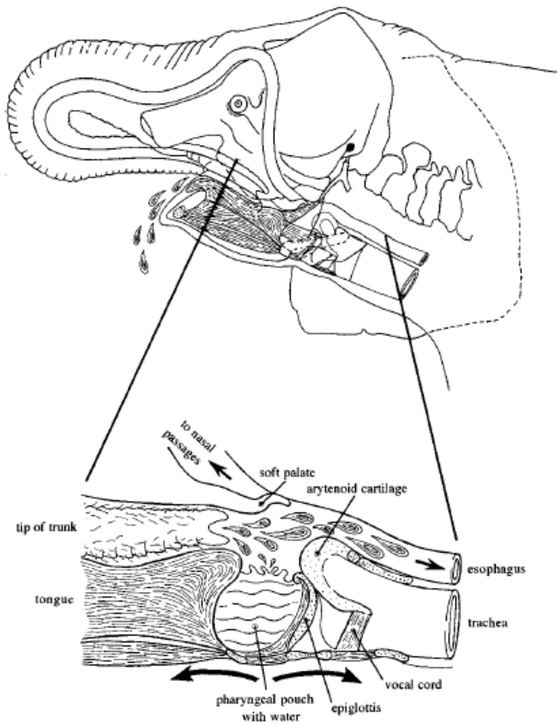


Figure 3 Illustration depicting the location of the pharyngeal pouch and how it is hypothesized to be filled during drinking (taken from Shoshani (1998)).

In contrast to other mammal species, the elephant´s lungs are not separated from the thorax wall by a pleural space. The latter is physiologically filled with connective tissue in elephants, which means that the lungs are firmly connected to the chest wall and its movements. Hence, chest wall movement should always be allowed in elephants during sedation/anesthesia and restricting positions (e.g. sternal) need to be prevented. This peculiarity can be explained by the enormous forces on the lungs during swimming and diving bouts (West, 2002). Without the more rigid connection between the lungs and the thorax, elephants might not be able to breathe when under the water surface (West, 2002). Before its description, this unique anatomical feature had been a source of confusion for pathologists.

As is the case in other species belonging to the superorder *Afrotheria*, the liver of elephants produces a fluid composed mainly out of bile alcohols. This is in contrast to most other mammals where bile acids account for the major part of bile (Hagey *et al.*, 2010). With respect to several reports on the occurrence of gall stones in elephants, it can be speculated whether bile composition presents a predisposing factor for this pathology (Agnew *et al*., 2005; Decker & Krohn, 1973; Jarofke, 2007; Pagan *et al*., 1999). A gall bladder is absent in the African as well as the Asian elephant (Eales, 1929; Mariappa, 1986).

Details on the digestive physiology of an elephant are given in the chapter **2.2 Feeding**.

While standing the limbs of an elephant look columnar and for quite a long time this was considered to explain why elephants are not able to run and jump. More recent research has demonstrated a rather large mobility, particularly in the distal joints (Ren *et al*., 2008) (Fig. 4). Although the high speed motion in elephants is lacking an aerial phase and may not fulfill the criteria for "run", elephants may reach maximum speeds of around 25 km/h (Genin *et al.*, 2010; Hutchinson *et al.*, 2003).

A) B)

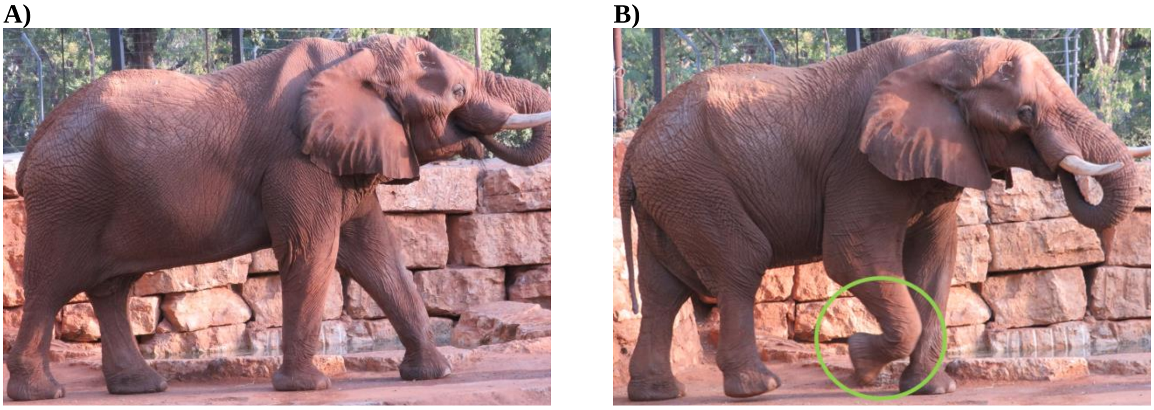


Figure 4 Although elephant legs look quite columnar when standing (A), in particular the distal joints show a rather large range of motion (B). © *Christian Schiffmann*

Having the largest brain of all terrestrial mammals (Fig. 5), elephants show an excellent retentiveness. The latter is pronounced in long-term, extensive, spatial-temporal and social memory (Hart *et al.*, 2008).

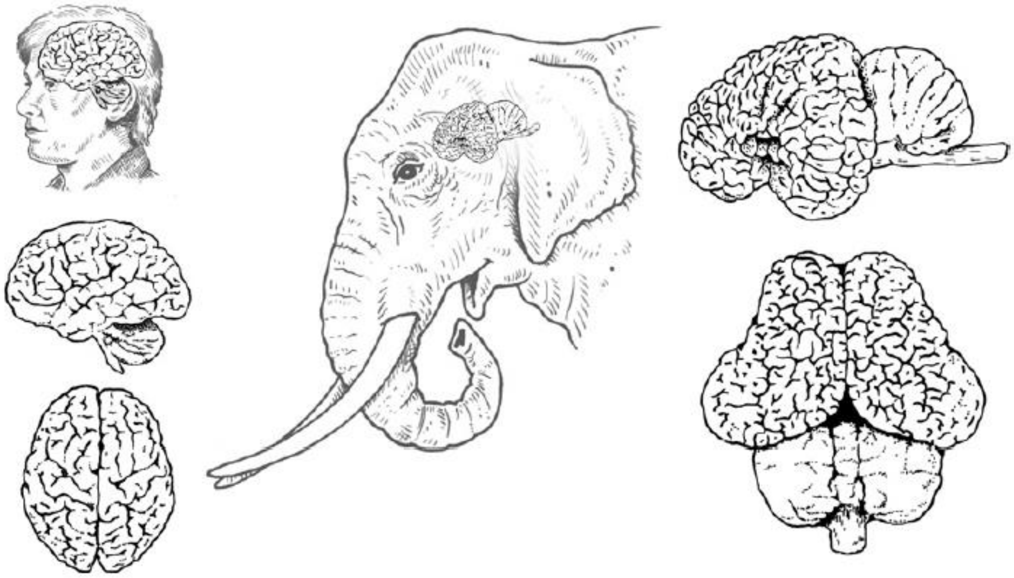


Figure 5 The relative size, shape and surface structure of the brain in humans and elephants (taken from Hart *et al.* (2008)).

Elephants possess a sophisticated cognitive system which presents the basis for intra-species communication. Details on these capabilities are given in chapter **1.8 Behaviour**.

1.4 LONGEVITY

It is generally accepted that life expectancy of elephants in the wild is from 40-70 years (Nowak, 1991; Kingdon, 1997). A recent analysis of female African elephant longevity and reproduction rates in Amboseli, Kenya conducted over a 40 year period found mean longevity to be 34 years (median 37,9 years) with 95% of females dying by 65 years (Lee *et al*, 2016), while previous studies had found median longevity of wild females to be 56 years for African elephants (also Amboseli, Clubb *et al.*, 2008) and 41,7 years for Asian elephants in the logging industry (Myanma Timber Enterprise, Clubb *et al.*, 2008).

Earlier research has demonstrated that average life expectancy of zoo elephants is similar to this (Wiese & Willis 2004). In Asian elephants living in European zoos, an average life expectancy of 47,6 years has been calculated while estimates for African elephants are less robust due to less data (Wiese & Willis 2004). A more recent analysis of long-term data on Asian elephants within the EAZA Ex-situ Programme found average life-expectancy to increase to 26 years for males and 35 years for females with the oldest living individuals in the EEP currently being 60 years (male) and 68 years (female) (Schmidt & Kappelhof, 2019). However, these results are skewed by the mortality of both Asian elephant males and females in first years of their life i.e. by data that are not always available for wild populations.

FIELD DATA

1.5 CONSERVATION STATUS/ZOOGEOGRAPHY/ECOLOGY

Population, Habitat and Distribution

For detailed updates on the population status and distribution of elephant species, please refer to the species assessment reports compiled by the IUCN Red List (**https://www.iucnredlist.org/**). Summaries of current data and other relevant references are provided below.

Elephas

Recent estimates suggest that the free-ranging elephant population currently numbers between 30,000 and 50,000. They are found across 13 countries, although nearly 60% of the population are found in India (Choudhury *et al*., 2008).
Wild Asian elephant populations are extremely fragmented and are facing a dramatic decline in numbers due to reduction and fragmentation of their forest habitat and the pressure from human population growth: 20% of the world's human population is currently living within the Asian elephants' range (*CoP17 CITES: Monitoring the Illegal Killing of Elephants (MIKE)*, 2017). The species is listed as *endangered* on the IUCN Red List.

Asian elephants are a generalist species found in a wide range of habitats including grasslands, evergreen forest, deciduous forest, scrubland and cultivated areas. Similarly, they are found at a range of altitudes from sea level to 3000m, although examples of beyond this range are known (Choudhury *et al*., 2008). Figure 6 shows the current distribution.

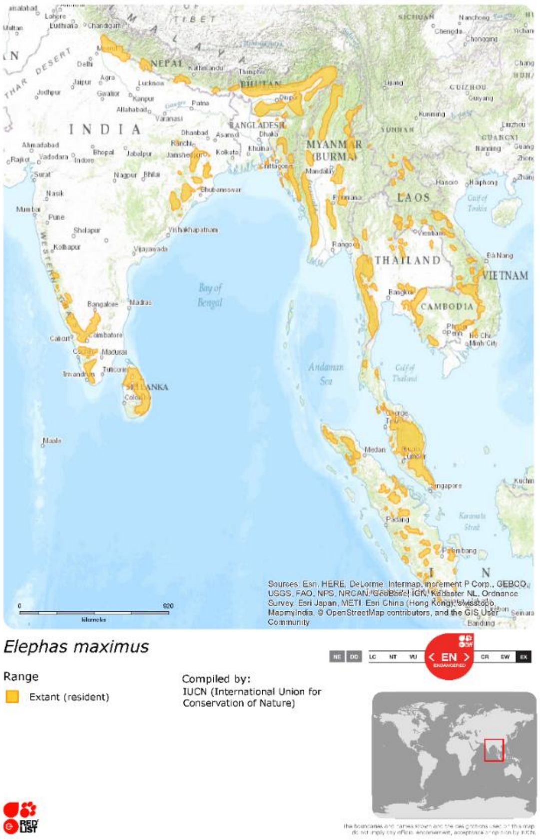


Figure 6 Current distribution of the Asian elephant (Choudhury *et al*., 2008)

Loxodonta

The IUCN African Elephant Specialist Group produced a status report in 2016 with numbers acquired, where possible, from each African range country reporting a continental decline in numbers of African elephants. The authors estimated that the total population of free-ranging African elephants currently stands at 415, 428 animals in areas surveyed, but the actual number across the full range is expected to be higher (Blanc *et al.*, 2016). Fig. 7 shows the present distribution. The species is listed as *vulnerable* on the IUCN Red List.

The two currently accepted species of African elephants are the savannah (*L. africana*) and forest (*L. cyclotis*) elephants. The savannah elephant resides on the savannah and grassy plains of East and South Africa, whilst forest elephants inhabit forested regions of Central and Western Africa.

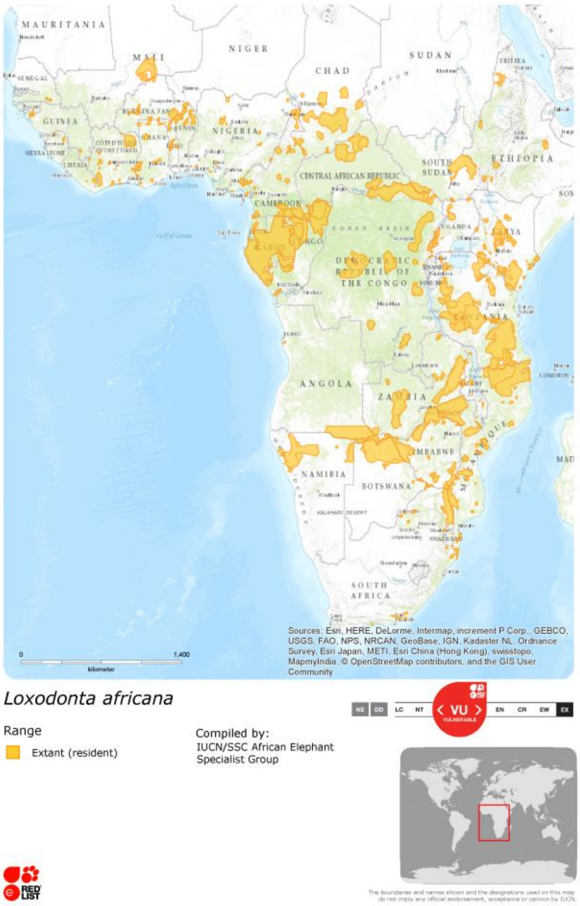


Figure 7 Distribution of the African elephants (Blanc, 2008)

Conservation and Protection

At the time of writing, the Asian elephant is categorised on the IUCN Red List as *endangered* due to population reduction resulting from habitat loss, fragmentation and human population pressures. The African elephant is listed as *vulnerable* on the IUCN Red List (Blanc, 2008). The most critical problems facing African elephant conservation are lack of financial resources for conservation management and growing human populations. Expansion of agricultural activities causes increased degradation and destruction of elephant habitat. This is most evident in West Africa which has the most fragmented elephant ranges. Over 40% of elephant range is in central Africa but only 10% of the area is protected. This region is forest habitat and supports the main population of *L. cyclotis.*

In 1989, the African elephant was listed as Appendix I on CITES which effectively placed a complete ban on the international trade in ivory and ivory products. However, in 1997 the species was down-listed to Appendix II, but only in some Southern African countries. This was, and is, somewhat controversial (Sharp, 1997) and currently, the African elephant is listed as CITES Appendix I, except for Botswana, Namibia, South Africa and Zimbabwe where it is Appendix II with certain restrictions including quotas and permit controls.

Elephant poaching and illegal wildlife trade

CITES has two monitoring systems for elephant trade: MIKE (Monitoring Illegal Killing of Elephants), which is the approved instrument for tracking the situation across Africa and Asia, and ETIS (Elephant Trade Information System), which is the designated system to monitor illegal trade in ivory and elephant products. ETIS was designed by TRAFFIC – a non-governmental organisation working on wildlife trade in the context of both biodiversity conservation and sustainable development (see **www.traffic.org**).

The ivory trade is one of the major causes of decline in elephant populations. Ivory has been a valued material from time immemorial and humans have been making and trading in ivory artefacts for some 10,000 years. Poaching for ivory is a problem for both African and Asian elephants, although it is probably greater for the African elephants as females also have tusks. It was estimated that in the early 2010s around 30,000 African elephants were poached illegally each year for their tusk to feed ivory demand in Asia (with 75% of the market being Chinese tourists in Hong Kong, Vietnam and Laos) (Save the Elephants, 2017). This led to a pledge by China and the US to end domestic trade in ivory in 2015 (TRAFFIC, 2015). In 2017, the domestic ivory trade was banned in China. The US introduced a near total ban, and Hong Kong, which is one of the largest city markets for ivory, announced a timetable to close its domestic market followed by a bill to ban ivory trade by 2021. In the UK, The Ivory Act 2018, which bans trade except in very few situations, is expected to come into force in early 2020 (UK Government, 2018). The Ivory Alliance, a coalition of political leaders, conservationists and celebrities dedicated to tackling illegal ivory trade, was also established in October 2018.

Culling (including through illegal poaching or through trophy hunting) can have a significant effect on social organisation and behaviour. Animals with larger tusks are often selected first, which in the past has resulted in a depletion of males and a drop in fecundity of females (Dobson & Poole, 1998). Additionally, in heavily poached populations, small family units have been seen comprising of abnormally high numbers of calves of similar ages and calves from heavily poached populations join unrelated females (Nyakaana *et al.*, 2002), both of which may impact on normal social behaviour.

Asian and African elephants are both listed on Appendix I of CITES. As per Article III of the Convention, Appendix I listed species can only be imported under strict conditions to ensure that such an import is not detrimental to the survival of the species, that the proposed recipient of a living specimen is suitably equipped to house and care for it, and that the specimen is not to be used for primarily commercial purposes.

In exception to the above, the African elephant populations of Zimbabwe, Botswana, Namibia and South Africa are listed on Appendix II, with the following annotations; *for the exclusive purpose of allowing trade in live animals to appropriate and acceptable destinations for Botswana and Zimbabwe and for in situ conservation programmes for Namibia and South Africa*. Due to the Appendix II listings of these populations, less strict conditions for importation apply from these populations. In light of concerns over the trade in African elephants from these populations, in particular Zimbabwe, moving to zoos in Asia, mostly China, that are thus not subject to the stricter rules applying to Appendix I species and populations, the CITES Parties approved a number of changes to CITES Resolution 11.20. The most important change is related to the interpretation of what are considered to be *appropriate and acceptable destinations* for receiving African elephants from theses Appendix II listed populations, which are defined as having *in situ conservation programmes or secure areas in the wild within the species' natural and historical range in Africa*. There are only very limited exceptions with strict provisions which only apply in exceptional circumstances. For more details please refer to **www.cites.org**.

In April 2017, the Asian Elephant Range State Meeting took place (facilitated by the IUCN SSC Asian Elephant Specialist Group and financially supported by several EAZA Zoos including Zurich, Antwerp, Emmen, Amsterdam and Rotterdam and was attended by representatives from range countries of Asian elephants. This resulted in the signing of the Jakarta Declaration for Asian Elephant Conservation with the aim to protect and conserve Asian elephants ("The Jakarta Declaration for Asian Elephant Conservation Jakarta, Indonesia"). One of the goals noted "that while elephant conservation is primarily a national responsibility, there is an urgent need to synergize national actions with international cooperation amongst the Range States for the long-term conservation of Asian Elephants. The reversal of the crisis facing Asian Elephants is additionally dependent upon political, financial, and technical support from the international community" (The Jakarta Declaration, 2017).

Sustainable use of elephant populations

Some countries have argued for a sustainable, controlled trade in elephant products (i.e. using elephants at a rate within their capacity for renewal which should not cause population level decline). Several African countries have protested against CITES ivory bans, arguing it should be possible to sell ivory legally through sport and trophy hunting in order to put financial revenue back into local communities and conservation through communal resource management (Bowles, 1996; Kock, 1996). In 2013, the Collaborative Partnership on Sustainable Wildlife Management (CPW) was established and is made up of 14 international organisations, which promotes conservation and increased cooperation and coordination (for full details see **CPW**).

However, for sustainable utilisation to work, strict control measures must be enforced. In 2015, The Global Environment Facility launched a global partnership programme for wildlife conservation. This programme works with governments of participating countries to strengthen opportunities for local communities to benefit from "healthy" wildlife and sustainable use, and to stop trafficking and demand for (il)legal wildlife products (full report available **Global Partnership on Wildlife Conservation and Crime Prevention for Sustainable Development**).

Human-elephant conflict

Definitions of the term vary (Inskip & Zimmermann, 2009), but a broad definition of human-elephant conflict (HEC) is "any human-elephant interaction which results in negative effects on human social, economic or cultural life, on elephant conservation or on the environment" (Parker *et al*., 2007).
HEC is a major intercontinental conservation problem (Hoare, 2000). Both African and Asian elephants are involved in HEC which occurs across much of their range (Hoare, 2000; Sitati & Walpole, 2006) and conflicts seem to be increasing (Naughton *et al.*, 1999). Increasing human population, expanding agriculture, war and political instability are the main causes of direct conflicts as humans and elephants compete for land (Kushwaha & Hazarika, 2004; Parker *et al*., 2007).

Conflicts between elephants and people can result in crop and property damage, economic losses, human injuries and fatalities, retaliatory killing of elephants and more indirect impacts such as stress, impaired freedom of movement and knock-on effects in terms of healthcare or education (Hoare, 2000; Dublin & Hoare, 2004; Parker *et al*., 2007). Consequently, in many areas HEC is a serious local political issue (Hoare, 2000), often undermining regional conservation and welfare initiatives (Naughton *et al.*, 1999). Researchers are attempting to predict HEC hotspots (Chen *et al*., 2016) and look at occurring trends (Pozo *et al*., 2017).

Some of the main methods currently being utilised to manage HEC are listed below.

Elephant-focused methods:

Physical barriers, elephant deterrents, aversive behavioural conditioning, habitat management, elephant population management and removal of individual conflict animals (Nyhus *et al.*, 2000; O'Connell, 2000; Sitati & Walpole, 2006; Parker *et al*., 2007; Hoare, 2012).

Human-focused methods:

Education and awareness, land use planning, compensation schemes, alternative livelihoods, and community wildlife benefits (Tchamba, 1996; Johnsingh & Williams, 1999; Nyhus *et al.*, 2000; Zhang & Wang, 2003; Parker *et al*., 2007; Fernando *et al.*, 2008; Bandara & Tisdell, 2005; Dickman, 2010; Olsson, 2014).

Zoo in-situ conservation support

Many EAZA zoos holding elephants are directly contributing to *in situ* conservation projects. For a list of past and current projects, see Elephant *in-situ* conservation projects supported by EAZA zoos available on **Elephant TAG SharePoint**.

1.6 DIET AND FEEDING BEHAVIOUR

Both elephant species are herbivores and consume a wide variety of plant material including grasses, leaves, twigs, fruits, barks, herbaceous material and soil (Kabigumila, 1993; Sukumar, 1990). A thorough review of diet breakdown, feeding behaviour, seasonal variation and summary data on broad nutrient ranges in natural diets for African elephants (*Loxondonta africana*) is covered in Sach *et al*. (2019). Variance between species does occur, with Asian elephants consuming a greater proportion of grasses in the diet when available (Cerling *et al*., 1999; Sukumar, 1990).

Although described as generalist herbivores, consuming over 400 species of plants, it appears populations may vary regionally and seasonally in their plant choice. However, it is clear that elephants are predominantly seasonal grazers and browsers with fruit, barks and soil being consumed as secondary food choices (Kabigumila, 1993). The natural diet is characterised by a high fibre content (crude fibre 30-50%) and a low-to-moderate protein content (crude protein 8-12%). Studies have demonstrated that passage of food through the elephants' digestive tract is rapid compared to other monogastric hindgut digesters such as horses. Total gut transit time is 11-46 hours (Bax *et al*., 1963; Hackenberger, 1987; Loehlein *et al*., 2003; Rees, 1982), and they have a correspondingly low digestive efficiency (Clauss *et al*., 2003; Hatt & Clauss, 2006, Clauss *et al*., 2007a). In summary, elephants are designed to eat large quantities of nutrient poor fibrous material which passes quickly through the gastrointestinal tract.

The body weight ranges overlap, however, Asian elephants (*Elephas maximus*) tend to be lighter than African elephants (*Loxodonta africana*). The weight range of wild adult Asian elephants is 1,800-5,000 kg compared to a range of 2,700-6,000 kg for adult African elephants (Wittemyer, 2011). Individual body weights are influenced by age, sex, health, food availability and, according to recent findings, by the molar state (Schiffmann *et al*., 2019a). Estimates of daily dry matter intake for an adult elephant are typically around 1-1,5% of body mass (Ullrey *et al*., 1997), however intakes vary according to the nutritional quality of the feed as well as environmental conditions, activity level, reproductive status and growth stage. These physiological factors influence energy requirements and therefore diet must be adjusted to meet energy needs in a captive setting.

Due to the limited research on vitamin and mineral requirements, animal nutritionists use domestic species as a model to make recommendations for captive exotic animals. For elephants, the National Research Council recommendations for the domestic horse are often used (Clauss *et al*., 2007b; Hatt & Clauss, 2006). Although using the domestic equid as a model has limitations, it is an extremely useful guide (Sach *et al*., 2019). Elephants have a single stomach and a short but voluminous hindgut fermentation chamber (similar to equids), inhabited by anaerobic bacteria and protozoa similar to those found in the rumen and reticulum of a ruminant. These micro-organisms digest plant fibre that otherwise could not be used since elephants, like other herbivores, have no fibre-digesting enzymes of their own (Ilmberger *et al*., 2014). Microbial fermentation of plant fibre in the hindgut provides the main energy source for these animals. They are adapted to eat complex plant fibres and thus in captivity, high fibre components must contribute a very significant part of their diet.

1.7 REPRODUCTION

Birth and development of young

As females of both species of elephants give birth within the family group, next to physical development, social development is of utmost importance from the very beginning in order to lead to successful breeding at a later stage. The cow-calf bond is strong. It is thought that alliances of females in families may enhance calf survivorship (Lee, 1987) and these alliances may be perpetuated by the long term relationships between allomothers and calves.

In Amboseli, Kenya, two births have been described (Moss, 1988), one to a primiparous female and the other to an older, experienced female. The visible onset of birth is characterized by the appearance of a bulge below the tail, which is the presence of the calf and/or sac in the birth canal, so the dam may have been in labour (hours) prior to this. Birth is then described to proceed rapidly. In the case of the primiparous female, the dam was agitated during the process and frequently scraped the ground with her forefeet. Other females may act to help remove the amniotic sac. Infants are initially unsteady on their feet, taking about 40 min to stand properly. The mother and other females use their forefeet and trunks to help infants stand; this unsteadiness remains for several weeks. The average weight at birth in African elephants and Asian elephants can be found in Table 5.

	African elephant		Asian elephant	
Variable	Male	Female	Male	Female
Birth weight (kg)	112.0 ± 3.5 (27)	95.1 ± 2.5 (30)	117.8 ± 3.5 (49)	118.1 ± 3.1 (44)
Birth height (cm)	93.4 ± 2.1 (16)	86.8 ± 1.1 (18)	95.9 ± 1.2 (19)	91.9 ± 1.3 (23)
Gestation (days)	634.5 ± 2.1 (10)	645.2 ± 3.0 (11)	660.6 ± 5.8 (16)	654.0 ± 4.3 (12)

The number of animals contributing to each mean is shown in parentheses (*N*). Each mean score is accompanied by the standard error of the mean [±SEM].

Table 5 Mean calf birth weight/ height and gestation period for Asian and African elephants (from Dale, 2010)

Infants must locate the teats between the mother's forelegs, unaided, and once located they will suckle several times an hour for two to three minutes at a time. The calf sucks and drinks using the mouth and must learn how to use the trunk to feed and manipulate objects. They gradually learn to use the trunk to collect food but are about four months old before they can eat a significant amount of solid food. It is normal to also ingest small quantities of dung from older animals in order to acquire the necessary microbes to aid digestion (Lee, 1991b).

Calves can be weaned at two years of age but usually suckle for four years or more. A female may allow an older calf to suckle at the same time as an infant (Douglas-Hamilton & Douglas-Hamilton, 1975) but in Amboseli, in two cases where an older sibling continued to suckle, the younger calf died. The survival rate of twins was also low in Amboseli (Lee, 1987). Cases of allosuckling have been observed but are very rare.

A study of growth curves in African elephants showed the typical sexual dimorphism in both foot length and shoulder height, with males growing more rapidly than females from birth onwards (Lee & Moss, 1995).

Adolescence in elephants

Adolescence is the time between weaning and puberty. In elephants, it usually covers an age from 5 to 10 years and is one of the most important stages of life in which young animals gather all the experience they will need to be able to survive and reproduce. Adolescence differs between female and male elephants. Whilst young cows stay in their natal group and acquire skills as babysitters and develop all maternal instincts, young bulls increasingly distance themselves from their family.

However, male calves must experience the complex social structure of the elephant herd and this must occur before sexual maturity is reached and they leave their natal herd. Younger bulls (up to 15 years of age) may stay in the vicinity of the family units, but older bulls tend to be more solitary and form bachelor groups, which are often unstable. These groups vary in size and age structure and enable them to become socially competent and sexually mature.

During adolescence the primary gender characteristics, being the ovaries in females and the testes in males, are growing along with general body development. Typical sex-specific changes in the body, or secondary gender characteristics, only occur with the onset of hormonal activity during puberty. Tertiary gender characteristics, such as sex-specific postures and behavior, develop individually during later stages of life. Adolescence ends with the final growth and reaching puberty.

Puberty in elephants

Puberty is defined as the age when the gonads have reached full function. In females, this is indicated by follicle growth and first ovulation in the ovaries, and in males, the production of viable sperm in the testes. Onset of reproductive ability is hormonally mediated and triggered by a number of factors, as shown in Figure 8. Apart from age, size, weight, body condition and health of an elephant, different external factors may have an effect on the onset of puberty. Food availability and season are the most important in the wild, whereas in captivity, stress levels have a higher impact because elephants are more restricted in space and unable to escape from difficult situations. Olfactory, acoustic, visual and tactile stimuli also contribute to kick-start the hormonal cascade controlling reproductive physiology.

Given good health and body condition of an animal, the hypothalamus is eventually responsive to these factors and starts to secrete gonadotropin-releasing hormones (GnRH) which induce the pituitary gland to produce gonadotropins, follicle stimulating hormone (FSH), luteinizing hormone (LH) and lactotropic hormone (LTH or prolactin). Whilst FSH and LH act on ovaries and testes, LTH directly affects mammary gland development. Under the action of gonadotropins, namely FSH, follicle growth starts in the ovaries, which are the source of estrogens during the follicle phase of the ovarian cycle. After induction of ovulation by LH, the former follicle cavity turns into a corpus luteum which produces progesterone. Progesterone is secreted throughout the luteal phase of the cycle and during pregnancy. In males, FSH acts on the testes and induces spermatogenesis in the Sertoli cells, whilst LH stimulates the Leydig cells to produce testosterone.

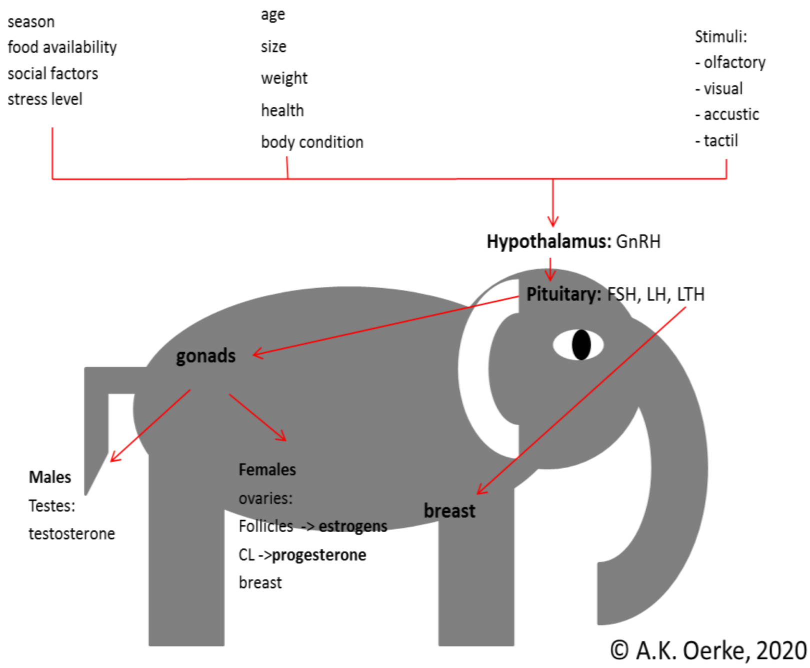


Figure 8 Factors affecting onset of hormonal action at puberty

Both sexes of Asian and African elephants generally reach puberty between 8 and 12 years of age. During this time, the complicated system of hormonal control of reproduction is working, yet animals may not be able to reproduce. In the wild, this is mainly due to social regulation. Young females are protected by their herd and it has been observed that mothers actively participate in the choice of whether or not mating is to occur, and if so, which bull may be allowed to mate them (Moss, 1988). Females need to learn about the mating procedure and must be physically able to withstand a gestation period of nearly 2 years. Hence breeding is dependent on physical condition and rarely occurs before the age of 10 years in the wild. Males need to learn to be able to detect estrus in females and perform the necessary behavioral repertoire to show dominance and make them suitable for mating. They must also be able to mate-guard the estrus cow until the fertile period is over and, at the same time, compete with other bulls that are seeking for breeding opportunities. Young Asian elephant males experience their first musth within 14 – 18 years of age (Eisenberg, 1980), whereas young African elephant bulls have not been seen in musth below an age of 20 and rarely get an opportunity to mate until they are at least 25 years old (Poole, 1989a).

It is important to note that environmental conditions can alter the age of puberty in both species. If African elephants are living under comparable conditions, population density is directly linked to the age at which young females give birth for the first time. In populations with high density, puberty is shifted to a later age. Laws (1969) found that in the Mkomazi Reserve in Tanzania, with an elephant density of only 2 animals per square mile, females reached puberty within 12 years of age, whereas in the Budongo Reserve in Uganda, with an elephant density of 7,5 animals per square mile, puberty was delayed to 22 years of age. At the same time, this effect might be compensated for in areas with high food availability. Douglas-Hamilton & Douglas-Hamilton (1975) reported in Lake Manyara, Kenya, even with an elephant density of up to 14 animals per square mile, there was still an onset of puberty at 11 years of age. Moss (1988) therefore concluded that elephants are able to respond to environmental changes in a highly flexible manner. In Amboseli National Park, Kenya, where elephants live at a density of only 3 animals per square mile, Moss observed that young cows, usually sexually mature with 12 years, shifted the onset puberty by 2 years after a period of heavy drought to an age of 14 or15 years of age.

Sexual maturity and gestation

Elephants have an extended lifespan, and therefore reach sexual maturity later in life as compared to most mammalian species. One study revealed that in female African elephants the mean known age at first reproduction was 13.8 years in the wild (Lee *et al*., 2016). Another study on Myanmar timber elephants demonstrated that females showed low fecundity initially following sexual maturity, with a rapid increase and peak at age of 19 (Hayward *et al*., 2014). Note that animals under human care can reproduce earlier than the average age for the given species.

Females usually produce their first calf approximately two to three years after their first ovulation. Gestation is 20–22 months after which a single calf is born (Estes, 2012). There is a low rate of twinning, around 2% (Douglas-Hamilton & Douglas-Hamilton, 1975). Elephants can exceptionally produce calves up to an advance age classes (e.g. 65 years recorded for African elephants in Amboseli) but show significant reproductive declines in older age (e.g. 40 years for Amboseli African elephants) (Lee, 1991a; Lee *et al.*, 2016). Calving intervals range from three to nine years, with an average of four years (Lee, 1991a), although there is variation between regions (Kurt, 1974; Moss, 1983; Sukumar, 1993).

Wild bulls mature between 12-14 years of age, which would seem to correspond to the female timing. However, a study regarding wild African elephants demonstrated that males were not able to mate successfully with oestrus females until about they were 25 years old; the majority of calves were sired by in-musth males in the oldest age groups (Poole, 1989b).

Seasonality

The breeding season of elephants is not easily defined, and months of conception vary from year to year. Elephants are not physiologically forced to breed seasonally, but some evidence of seasonality has been recorded. For example, conception has been linked to wet periods in some areas (Douglas-Hamilton & Douglas-Hamilton, 1975), but was not observed in others (Nowak, 1991; Sukumar, 1993; Eisenberg & Lockhart, 2011; McKay, 2011). However higher quality foods, usually available after rains, result in improved body condition and increase the likelihood of ovulation occurring (Lee, 1991a) and it is likely that periods of nutritional stress decreases the likelihood of cows ovulating. This may help explain the phenomenon that some years appear to have resulted in a very low number of conceptions (Sukumar, 1993). A recent review of breeding seasonality in African and Asian elephants reports them to be “long-day breeders” with underlying photoperiodic cueing (Hufenus *et al*., 2018). In order to encourage natural conception these findings can be of practical relevance.

Musth

Both Asian and African male elephants exhibit a condition known as musth. When in musth, males have elevated levels of testosterone (Cooper *et al*., 1990), aggression and reproductive activity (Poole & Moss, 1981; Dickerman *et al*., 1997; Poole *et al*., 2004, Poole *et al*., 1984; Niemuller & Liptrap, 1991). The discharge of fluid from the temporal gland increases and is continuous (in Asian elephants); the penis develops a greenish colouration and dribbles urine; they also vocalise frequently in a particular manner (musth rumble). It has been shown that males emit volatile compounds from the temporal

gland and within their urine which may inform other bulls and cows of their condition (Rasmussen *et al.*, 1990; Rasmussen, 1997).
There are many questions as to the function of musth (Wingate & Lasley, 2001) and a suggestion that it may be a relatively recent phenomenon in the African species (Rasmussen *et al.*, 1990). It is proven that the small number of older bulls in the competitive state of musth are the most successful sires; however, non-musth males sired 20% of genotyped calves, and 60% of mature bulls (>20 years old) were estimated to have sired offspring during a 5-year study period in wild African elephants (Rasmussen *et al*., 2008). In Amboseli, very few males under 24 years of age have been seen in musth and bouts of musth among individuals of the 25-35 age group are short and sporadic, while older males have longer bouts sometimes lasting several months (Poole, 1989b). This work has also shown that the number of males in musth correlates closely with the number of oestrus females and is highest during the rainy season, when females are more likely to ovulate. Both Asian and African bulls settle into a regular cycle of musth with increasing age.

Apart from the interesting dynamics of musth and non-musth males in the similar area, it must be concluded that in a captive situation, there is no relation between whether a bull can sire offspring and having elicited signs of musth.

1.8 Behaviour

Of the terrestrial animal species, elephants have the greatest volume of cerebral cortex available for cognitive processing (Hart *et al*., 2001). This exceeds that of any primate species and allows this long-lived species to develop many skills involving learning and memory, including storing information on conspecifics and the environment it inhabits.

Activity

Elephants spend about 16 hours a day feeding. They sleep for four or five hours within a 24 period, sometimes lying down. Therefore, although primarily diurnal, they are active in the hours of darkness. Elephants perform remarkable movements and postures; they can roll, kneel, squat, sit on their haunches and climb up very steep slopes (Estes, 2012). However, they cannot run or jump but only walk at varying speeds.

Social organisation

The most basic elephant social unit is a mother and her offspring. A family unit is a group of related females, consisting of a mother and young, including her own mature daughters and offspring (Moss & Poole, 1983). The matriarch, who will be the oldest, largest and possibly even post-reproductive female, sets the activity, direction and rate of movement of the herd. The leadership and experience of the matriarch is thought to be of great importance and it has been suggested that there may be higher per capita reproductive success in groups led by older females (McComb *et al*., 2001).

In Manyara, Tanzania, the average size of a family unit was 10, and these units group together to form larger kinship or ‘bond’ groups, which may consist of as many as 50 animals. When the number of elephants increases beyond a critical number (which depends on several factors), a new matriarchy (family unit) splits off but will remain as part of the same kinship or bond group. These kinship groups probably form part of a larger association, often termed ‘clans’, which would explain observations of gatherings of up to 100 animals or more (Eltringham, 1982).

The current suggestion for elephant social organisation therefore includes a core group of a family unit and these units are further associated together in bond groups. Bond groups are probably comprised of closely related individuals resulting from the fission-fusion of family groups. Bond groups come together in clans, which combine to form sub-populations and then the population of an area. Although most detailed work on social organisation is from studies of African elephants, clans have also been identified in Asian elephants (Sukumar, 1993) and their families do also split and come together (McKay, 2011).

A study in Sri Lanka found bond groups of 22-58 animals, which split into family groups comprised of between one and four reproducing females (Heine *et al*., 2001). Other data gathered in Sri Lanka shows a splitting of groups of females with young infants (a nursing unit) from groups with juveniles (a juvenile care unit) (McKay, 2011). This appears to be due to females with infants at heel associated more with other, similar females but between these groups there is still a degree of flexibility and interchange of individuals (Kurt, 1974; McKay, 2011).

Smaller group sizes were found in the rain forest of Malaya and Sumatra (Eltringham, 1982). Santiapillai & Supahman (1995) found group sizes of 4-8 animals in the forests of Way Kambas in Sumatra but larger aggregations of up to 45 animals were found in areas of grassland, with smaller groups being more common in the dry season. In general, herd sizes differ between populations, seasons, and habitat types, e.g. desert elephants disperse over large areas in relatively small groups and probably rely on infrasound to communicate between groups (Lee, 1991b).

The most detailed study of relatedness between animals in wild Asian elephants was carried out using mitochondrial DNA studies from the dung of animals in Sri Lanka, as well as direct observations (Fernando & Lande, 2000). Four groups were observed in detail, ranging in size from 7 to 19 animals. Solitary ranging females were observed and there was an overall lower level of association between group members compared with African savannah elephants. The genetic analysis showed that all individuals within a social group shared the same mtDNA haplotype and that they all must have descended from a single female in the recent past. Groups with overlapping ranges maintained their maternal genetic identity, suggesting that females do not transfer between groups.

Less is known of the social organisation of African forest elephants, but group size is much smaller, averaging 2 to 4 individuals. Groups seem to consist of single mother family units (Turkalo & Fay, 1995), with older males being solitary. It appears, from evidence to date, that elephants living in forest and woodland habitats may tend to split into smaller units more frequently than those in savannah and have less direct inter-group contact.

The home range of a cow herd is much larger than the area used by a male, and cow herds will pass through the home ranges of several different males, although data from Sri Lanka suggest that family groups with young calves may have smaller home ranges (Kurt, 1974; McKay, 2011).

Sexual behaviour

Female Asian elephants show an oestrus cycle length of 14-16 weeks and African females of 14 weeks (Oerke *et al.*, 2000). This is a relatively long oestrus cycle with a brief receptive period of 2-7 days (Rasmussen & Schulte, 1998). A considerable amount of research has taken place to obtain detailed information on the elephant ovarian cycle since early work in the 1980's (Plotka *et al*., 2005) and this is reviewed in Hodges (1998).

Female elephants advertise forthcoming ovulation to males by characteristic behaviour patterns which include: oestrus walk (head held high, eyes wide open and tail may be raised), chase, mounting and consort behaviour (Moss, 1983); these patterns are shown for two to six days. Asian elephant females also advertise a forthcoming ovulation by releasing (Z)-7-dodecenyl acetate in urine during the pre-ovulatory phase to signal to males their readiness to mate (Rasmussen & Schulte, 1998), however this compound has not been found in African elephant females (Riddle & Rasmussen, 2001). Females also produce a unique vocalisation when in oestrus (Poole, 1999) 'the oestrus call'. Thus, females can communicate their reproductive state by olfactory, visual and auditory means.

It has been shown that females can distinguish, by olfaction, between musth and non-musth males as well as the maturity of musth males (Rasmussen *et al.*, 2002), and they are more responsive during the follicular stage of the oestrus cycle (Schulte & Rasmussen, 1999; Ganswindt *et al.*, 2005). Oestrus females enter consortship with a musth male who guards her from copulatory attempts from lower ranking males. Males in musth rank above non-musth males in agonistic interactions and females prefer older musth males (Poole, 1989a). In the Amboseli population, males did not compete successfully for females until they were 25 years or older and larger musth males over 35 years of age were more frequently seen guarding females mid-oestrus. Males may check the reproductive state of females by putting the trunk tip to the vulval opening and then inserting it in the mouth, the elephant equivalent of flehmen.

Copulation is rapid, with the male mounting the female from the rear and both mounting and copulation together lasting on average 45 seconds (Estes, 2012). Females may emit a low-frequency post-copulatory call (Poole, 1989b).

Communication

Elephants have a complex repertoire of communication, which includes touching, vocalising, olfaction and body postures. Elephants are tactile animals; family members lean on each other and frequently touch eachother with the trunk. In a greeting ceremony, the lower ranking animal inserts its trunk into the mouth of another and trunks are also held out to other animals in greeting. The trunk is also the olfactory organ, picking up chemical signals from other elephants. Many postures are used in communication e.g. spreading the ears and holding them forward is normally a threat and various trunk movements may signify submissive behaviour (Langbauer, 2000). The combination of posture, vocalisations and olfaction provide sophisticated means of communication.

Elephants produce calls in the larynx and some vocalisations are in the infrasonic range, below human hearing thresholds. The infrasonic calls have fundamental frequencies ranging from 14-34 Hz and sound pressure levels as high as 103dB. Low frequency sounds are subject to little environmental attenuation and can be audible to conspecifics several kilometres away, certainly as far as 2-4 km and perhaps further. Elephants make use of these calls for spatial coordination and in the search for mates (Poole *et al*., 1988; Langbauer *et al*., 1991; Langbauer, 2000; Stoeger & Baotic, 2016; Baotic & Stoeger, 2017; Baotic *et al*., 2018).

Elephants have four main sounds (Estes, 2012) but a great variety of pitch and duration within each one. Rumbling is the main form of distance communication and covers a broad range of frequencies, many infrasonic. Quiet rumbles, audible to human ears, are uttered as a herd feed. There are known to be over 27 different low-frequency rumbles (Poole, 1999). Elephants growl when greeting and their voices are individually recognisable. An increase in volume becomes a roar, and is used to threaten predators or man. Screaming is used to intimidate opponents and is the adult equivalent of the juvenile distress call, the squeal. Trumpeting is the sound of excitement and is produced by blowing through the nostrils hard enough to cause the trunk to resonate in a long, high-amplitude squeak. It is usually combined with growling and screaming. Trumpeting can range from an expression of alarm or a cry for help to a greeting call.

The musth rumble is a rumble emitted only by males in musth and the oestrus call is a cooresponding, distinctive rumble emitted by females in oestrus, with the latter being the same call that is emitted by the female post-copulation (Poole, 1999). Males are attracted by the oestrus call of females and likewise, females in oestrus are attracted by the call of musth males (Stoeger & Baotic, 2017).

Recent research in zoo conditions provided new insights into vocal communication and vocal learning of infant African elephants (Stoeger-Horwath *et al*. ,2007; Stoeger & Manger 2014).

Play

Play in young includes head-to-head sparring, trunk wrestling, mounting, charging and rolling. Calves tend to play with others similar in age, and calves over six months of age may form play-groups (Eisenberg & Lockhart, 2011).

Tool Use

Elephants exhibit tool use which, in the wild, can range from using grass or sticks to rub or scratch the body (Chevalier-Skolnikoff & Liska, 1993). There are also reports of elephants using branches as fly switches (Hart & Hart, 1994) and modifying an unsuitable branch so that it becomes useful (Hart *et al*., 2001). Elephants have been observed to use tools in various contexts, including in skin care, feeding and drinking, threat and aggression, rest and sleep, and social interactions. Types and variety of use increases with age.

SECTION 2: MANAGEMENT IN ZOOS

All current holders are **strongly encouraged** to meet the following standards.

2.1 ENCLOSURE

Suitable enclosure design involves much more than simply consideration of size of space. Research shows "that the size of an exhibit plays a secondary role in supporting optimal welfare of zoo elephants. It is, however, important to consider space configuration as well as flooring" (Meehan *et al.,* 2016). The indoor and outdoor environment should be complex, positively challenging to the animals and must contain devices and structures which enrich the environment and encourage natural behaviours. When designing a new enclosure, collections must consult the EAZA Elephant TAG housing committee for advice.

For new facilities with plans for a breeding group, the following point is obligatory.

- All zoos with a breeding herd must have 3 separate facilities with appropriate outdoor paddocks. The aim is to follow the fission/fusion nature of the ever-evolving elephant group e.g. long-term housing of breeding group and breeding bull with flexible spaces to hold young males until age of dispersal (12+). Continual housing of bachelor groups if social issues develop (capacity to appropriately separate animals) should be possible.

Minimum requirements for enclosures are:

- to be complex enough to allow environmental choice and encourage exercise, e.g. multiple options that engage enrichment categories such as choice of multiple substrates, multi-level feeders, multiple feeding stations, varied topography (to stimulate exercise and provide visual barriers), water features, mud wallows, dust baths, and long-term feeding.

- to enable social choice, i.e. no dead-ends or areas where elephants could become trapped, large enough spaces for animals to get away from conspecifics, and spaces with visual barriers between conspecifics and/or public.

- to have multiple resources which should not be socially limiting, e.g. multiple areas providing shade, food and water so that all animals do not have to crowd around a single source. There also must be adequate resource provision, so animals are able to choose which to use.

- to reflect local weather conditions, e.g. larger, climate controlled indoor spaces available in regions where winters are long and cold.

- if possible, to enable free choice of indoor/outdoor access over the 24-hour period throughout most of the year except in periods of extreme weather (depending on the local climate). To prolong the period of free overnight access during colder months while preventing loss of heat from indoors, heavy plastic/rubber curtains should be installed over entry doors to the house. Care should be taken in herds with small calves, as mothers may not always recognise when calves are becoming cold and bring them indoors.

- to ensure keeper health and safety. This must be built into enclosure design from the onset.

- to have overnight visual monitoring techniques such as CCTV monitoring to record social relationships and behavioural patterns, with regular review of footage.

- to facilitate best practise in terms of husbandry (including Protected Contact management) and veterinary access.

Bulls require all the same housing resources as cows (e.g. bathing, topography, substrate, scratching, shelter and visual barriers). In addition, bulls require the following:

- All outdoor cow areas must be adjusted and safe for a bull (make it bull proof).
- Collections must have an appropriate separable area for bull for times of required separation. This may be through the bull’s choice, in which case keepers may monitor and physically separate the bull, or due to a non-breeding recommendation from the EEP. Additionally, the bull may need to be separated if the welfare of the female(s) becomes compromised.
- The bull area should be within a separate building to prevent frustration when females come into oestrus, which can cause ongoing social tension within the group. It may be necessary for bulls in musth to be kept out of a female and calf herd for management reasons when separation can be difficult due to heightened aggression and reduced response of the animal.
- Bulls should not be kept in long-term physical isolation and should have many opportunities for socialisation. Collections should run animals together where possible as social learning gained by calves from bulls is hugely important.

Stationing walls

To safely move the elephants around the facility, the provision of stationing walls or areas (Fig. 9) is a significant advantage. The stationing wall will provide very limited access of the elephants to the keepers but could also incorporate husbandry access ports for greater flexibility of access to the elephants by the keepers. The stationing walls allow elephants to be held away from door operations or away from each other without physically separating animals. The inclusion of significant lengths of stationing walls would be an advantage, particularly for larger elephant groups.

Figure 9 PC stationing wall

Elephant restraint device (ERD)

Restraint devices are large but narrow areas that can limit the movement of elephants if necessary (see Fig. 10). Changes in elephant behaviour, herd dynamics, individuals, and personnel, along with the potential of a catastrophic illness or injury, make an ERD a necessary piece of equipment as a safe way to carry out essential veterinary procedures with their bulls. An ERD restricts most—but not all—of the animal's mobility. Movement of the trunk, feet, and tail is limited but these extremities can still be used aggressively. Therefore, although the access for husbandry and medical care is safer, it is not risk free and handlers are exposed to the elephant, thus safe operation of an ERD requires trained, skilled staff. All facilities should have ERD enabling proper PC techniques.

Despite the many variations in design, there are a few basic elements shared by all ERDs. An ERD should allow safe access to all four feet, tusks, trunk, face, ears, both sides, hindquarters, and back by moving the animal or parts of the ERD. It may have a side that can be moved laterally to further restrain the animal. The ERD must open easily and quickly to free an elephant that has collapsed. It should also be able to comfortably contain an elephant for extended periods of time should the need arise for an ongoing or long-lasting veterinary or husbandry procedure.

The location of the ERD is important; it must be incorporated into the facility design and use must not be affected by weather. To encourage the animal to use the ERD, they should be designed so the animal can enter from either side and can walk through on a routine basis. The ERD should not be used solely for uncomfortable veterinary procedures as most of the animal's experience with the ERD should be positive. The ERD must be accessible to all animals.

Figure 10 ERD side view with ports

Modern ERD designs allow use of special attachments which can be used for semen collection, or give access to hind legs for blood sample collection(see Fig.11).

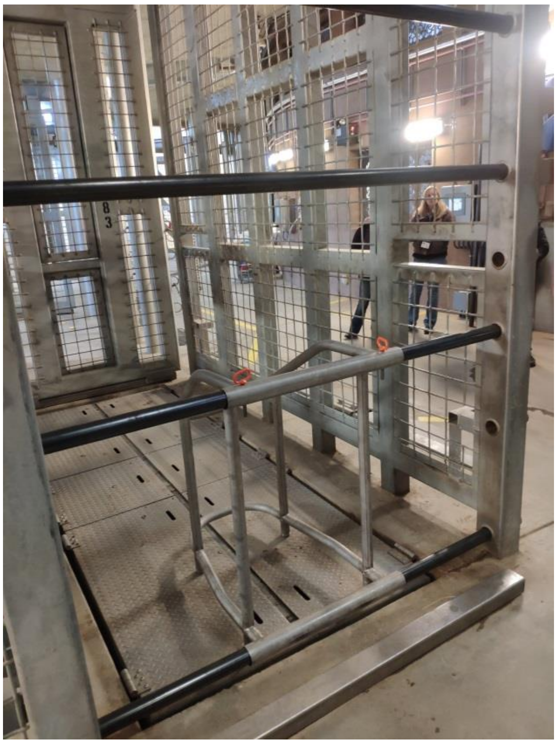


Figure 11 ERD with special attachments

Protected contact (PC) wall see chapter **2.6.2 General Handling**

2.1.1 Boundary

The main goals of barriers are safety and prevention of direct contact with the public and staff. Thus, barriers must be well maintained and escape-proof. The most common barriers are either concrete walls, gates/doors, electric fencing (secondary barrier if not steel ropes), and/or water or dry moats.

Concrete walls

If a tusked elephant has a tendency to dig at walls then it should be housed in facilities with smooth walls to prevent damage to both walls and tusks. The minimum height of the barrier is 1,9m for cows and 3m for bulls. However, it is recommended to use height of 3m everywhere to make the facility bull proof.

Gates and doors

Gate design is vital for the safety and well-being of both elephants and staff. They are either used for separation of a given area or,if adjusted, can serve as a supplementary PC wall as well.

Doors must be designed to be operated safely by staff, i.e. outside of the area within the elephant's reach. Safety corridors and stand-off areas should be 4m wide. Doors must be able to be quickly opened and closed and should have a stop capability to ensure tails/trunks are not injured and elephants cannot open half closed gates.

Gates and barriers should not have horizontal bars, which would allow elephants to climb. Bars should be either diagonal (sharp angles should be checked for safety and preferably avoided) or vertical. For safety of staff, it would be advised to use diagonal bars at place of close contact between animals and staff.

Engineering must be robust and any hydraulic system should have manual back-up and/or alternative power. Hydraulic gates and systems should not use standard hydraulic oil, as this is toxic to animals if it leaks, but a suitable vegetable-based alternative should be used.

Electric fencing

Electric fences (in form of thin wires) should be used as secondary barrier only as tusks do not conduct electricity. They should have sufficient voltage (suggested voltage is about 8000v @ 3.5 joules) and a fail-safe alarm system. There are several designs available that are very effective and communicate with CCTV cameras; in the event of the fence being compromised the resulting information will be relayed to a camera which will change its position to the area in question.

Moats

Dry moats with a hard surface have become obsolete as they pose a real threat of injury, especially to young elephants. They should be replaced in the long-term with interim plans made for getting out any animals that fall or are pushed in. Moats that are deep (> 1,4 m), narrow (< 3m) and hard bottomed should especially be phased out. Dry moats or "ha-ha" ditches should be wide enough for an elephant to turn in, and not deeper than 1,75m. The bottom surface must be soft and a ramp should be provided so that an elephant can climb out of a moat if necessary. For water moats (or pools) see chapter **2.1.3 Furnishing and maintenance – Bathing.**

2.1.2 SUBSTRATE

Housing substrates – indoor and outdoor

Substrate affects elephant health including respiratory health, foot health and overall wellbeing, through increased lying (Holdgate *et al.* 2016, Miller *et al.* 2016). Substrate is one of the easiest ways of increasing enclosure complexity. Whilst sand is one of the main substrates of choice, other substrates can provide points of interest and investigation, such as bark chip, clay, mud, grass and soil. The combination of surfaces (hard and soft) will help reduce foot/toenail overgrowth.

Indoor and outdoor substrates should:

- provide choice to the elephant, allowing elephants to explore and investigate a range of substrates within their enclosure. This can provide increased activity and the cognitive benefits of decision-making.
- provide a degree of flexibility and accommodate the vast body mass of an elephant. Flexible ‘soft substrate’ works to absorb impact, easing the pressure on the joints and feet.
- provide good drainage which is beneficial for respiratory, skin and foot health. Soft substrate (such as sand) can act as a “bio floor”, allowing drainage of urine and ensuring animals are not standing in their own quite aggressive urine or lying on wet/cold floors.

- provide opportunities for enrichment, such as digging/hiding food which can improve musculature.
- allow dust bathing which provides enrichment and is beneficial for skin health.

Hard floors

Should be used only in places where long standing of elephants is not expected to occur and the elephant's time in these areas should be minimised where possible. Concrete or rubber floors should have a non-abrasive but not smooth finish. Solid floors should be cleaned regularly and disinfected where appropriate and should provide appropriate drainage to avoid pooling of urine where elephants stand.

Sand floors

If used, type of sand should be considered. It should not be dusty, should drain well and not be able to compact (in the enclosure or gut). Sand that includes fine sand, silt or clay grades is likely to result in a dusty enclosure and compact into a solid floor. Sand grains need to be of a single size to reduce compaction. A grain with a round, rather than angular, shape will reduce compaction and will not be over wearing on the feet.

Sand depth should be of 0,8 minimum depth, but 1,5m is recommended. Daily maintenance of sand includes daily watering to prevent excessive dust. Sand must be regularly turned to prevent compaction and build-up of bacteria that can grow in anoxic conditions. Sand floors need to be easily accessed by specially designed heavy machinery (such as truck loaders), and purposely built concrete ramps are a must.

Where sand is retro fitted into enclosures, drains should be protected with permeable membranes and sand depths maximised where possible. Door runners should be subject to increased maintenance procedures to mitigate sand ingress. Alteration of door runners can be considered in preference to removal of substrates.

Benefits of sand include the ability to build up pillows or mounds which may be used by older animals to sleep against or aid older/arthritic elephants when getting up. Animals also can benefit from rolling/ playing in or on mounds and they can act as visual barrier. The drainage provided is beneficial to aid calves in standing up quickly post birth. Sand can be used in outdoor areas, in all weather, and can withstand extreme cold or wet weather (with appropriate drainage).

Grass

If possible (i.e. space available will not be significantly compromised with paddock rotation technique), allow grazing access for elephants, which prolongs foraging times.

2.1.3 Furnishing and maintenance

A complex and variable enclosure is essential. The indoor and outdoor environment should be positively challenging and stimulating to the animals and contain devices, structures and terrain which provide variety and complexity to enrich the environment and encourage natural behaviours including, for example, moving around, grazing, dustbathing, bathing, scratching, digging and exploration.

Enclosures should contain as much of the following as possible:

- **Visual barriers** – should be available in the enclosure to enable elephants to get away from conspecifics within their group or between different groups (if multiple groups kept). This may be helpful to reduce aggression.
- **Topography** – a variety of slopes and terrains will encourage significant muscular activity, provide vistas for the elephants and allow elephants the choice of social interaction. Regular rejuvenation of topography will ensure that enclosure use is maximised.
- **Bathing** – there should be at least one water bathing option both indoors and outdoors. A pool is recommended both inside and outside and at least one pool must be present somewhere within the enclosure ideally with year-round access to meet the needs of all animals. The pool should have gentle entry slopes (not normally greater than 25 degrees) with non-slip surfaces to encourage all animals to use. Pools should not have vertical sides such that they pose a danger to animals when empty. Pools should have multiple entry and exit points to prevent animals from becoming trapped and to reduce potential social tension. The size of the pool should be large enough to accommodate all elephants in the group without causing social conflicts. The pool should be deep enough to allow for bathing behaviour and the full immersion of all adults together. Other water bathing options include waterfalls or sprinklers. Keeper-led elephant washing may have a role to play in skincare but does not replace free-choice bathing in a pool. Elephant preference will be to bath in clean water and provisions should be made for efficient filtering or regular water changes in pools to ensure use by elephants is optimised.
- **Other non-water bathing opportunities** - should be available including dust baths and or mud wallows. Mud wallows should have shallow sloped entry and be regularly refreshed. They should have relatively wet clay and a stable natural base to avoid nail damage. Clay should not be too thick to avoid young elephants getting stuck inside.
- **Shelter** – sufficient shelter areas (from rain, wind, sun etc.) must be provided to allow access without compromising social choice.
- Opportunities for **scratching and wallowing** must be provided e.g. rocks, scratching posts or trees and mud wallows. Scratching and dusting areas should be in close proximity to mud wallows and pools to maximise use by elephants.
- **CCTV** - should be installed to cover the majority of elephant enclosures and should have the possibility to review footage from the facility regularly to determine behavioural changes. This should include the ability to view in the hours of darkness using infrared emitters.

2.1.4 Environment

Indoor ventilation and air changes

Indoor housing with a substrate floor will inevitably produce large amounts of dust either intensively for short periods or cumulatively over a period of time. Ventilation is required to maintain air quality for animals and staff, and is usually provided mechanically via extractor fans, possibly with filters for key areas. Fans or air-conditioning should be considered for cooling in warmer summer months.

Indoor facilities should have as much natural lighting as possible. Artificial lighting in the indoor spaces should come on and off gradually so as not to suddenly plunge animals into darkness or into sudden light. Light levels should represent natural outside light and the use of supplemental UV light should be considered if animals are contained indoors for long periods of time.

Temperature

The inside temperature must be no less than 15 - 18°C (with respect to outdoor temperature). Usage of spot heaters (at least 21°C) to fulfil special needs of certain individuals is advised. Typical scenarios include newly born calves, geriatric animals or newly imported animals in a phase of acclimatization. Elephants can use outdoor enclosures from temperatures above 10°C (apparent temperature) without limits, 7°-10°C with free choice to use indoor enclosure. Due to their great body size, the cooling down rate of the body temperature is slow. However, the weak point could be the ears. Therefore, under windy conditions and below 7°C, time spend outdoors should be monitored. Animals of different ages might have different needs and special attention should be paid to calves under 2 years of age.

2.1.5 Dimensions

Elephants are large animals and thus always at the centre of debate of whether or not the species can be cared for properly in the confinement of a zoo environment. Of course, the more space one can give the better, but to quantify dimensions in a guideline is not so straight forward. Luckily, more and more zoos are facilitating research to provide data on how their animals use the exhibit space. For example, a GPS-based locomotion analysis of a bachelor group of young Asian elephant bulls in Zoo Heidelberg showed that a bachelor group of four in a 2500m² enclosure covered walking-distances comparable with data known of wild Asian elephants (Linti & Reichler, 2018)

The complexity and furnishing plays a more important role than simply the size of the enclosure. Group composition and group dynamics are other important factors to consider. Finally, the time spent in a particular stable or pen is another factor to take into account. Enclosures must be flexible and allow for animals to get away from each other if they choose, and for separation to occur where needed.

However, it is recommended that all indoor cow/herd facilities, where animals are locked in for a considerable period, provide at least 300 m² for each group of four (or fewer) animals, and for each additional animal over two years of age, increase this space by at least 80 m², provided that the indoor facility is a complex environment. Even in the most compatible herds, consisting of related animals, individuals have the need to be able to retreat.

It is recommended that the indoor facility for a mature bull provide at least 160 m² and should take into account their ability to reach vertically up to six metres, and up to eight metres if able to climb their front legs against a wall. Bachelor groups of adolescent bulls, even when unrelated, tend to stick together and even sleep side by side at night. As adolescent bachelor bulls grow older it is necessary to split up individuals for short periods (e.g. during night-time). The facility should then still be able to provide sufficient space for each individual until the animals are re-united.

Outdoor areas for adult bulls and cows (i.e. over two years) should provide all animals with a shared space of 3,000 m² throughout the year. The outside bull pen should be no smaller than 1000m², or 500m² in cases of separation lasting only for a maximum of a couple of hours a day. If possible, the bull should be allowed to roam with the cow herd.

Local weather conditions play a crucial role in determining the space requirements for elephants. When elephants have free choice between indoor and outdoor areas, elephants are happy to roam outside with temperatures just above freezing point, as long as they have a place to warm up and sleep. However, facilities with calves and/or debilitated herd members may have to lock animals inside (also see chapter **2.1.4 Environment**) at low temperatures. This means that throughout the EAZA region, one elephant will only have a stable without access to the outside area for at least half of the 24 hour day while another elephant, in another zoo, might have a relatively small stable, but permanent access to the outdoor area 24h/day.

2.2 FEEDING

2.2.1 BASIC DIET

Zoos have a duty to provide optimum nutrition to all animals in their care. Failure to do so may result in nutrition-related disease, compromised welfare, potential reduction in reproductive success, as well as reduced life expectancy. Formulation of an appropriate diet for captive animals requires both husbandry skills and applied nutritional science (Dierenfeld, 1997). Generally, in Europe, elephants are fed a diet of predominantly forage consisting of hay, grass and browse with added commercial pellets and some produce (Hatt & Clauss, 2006; Partington, 2012; van Baarlen & Gerritsen, 2012). Due to the quantity of food required by an elephant, feed costs are the second largest day-to-day running costs of an elephant herd , as evidenced in the UK (Sach *et al.*, 2019). Therefore, it is essential that zoos are feeding appropriate items of acceptable quality to their animals, in line with optimum procurement practise to maximise their financial spending. The major nutrition-related health problems facing captive elephants are obesity and colic, as well as individual cases of molar disorders and specific deficiencies, including calcium, zinc, vitamin E, iron and vitamin D (Hatt & Clauss, 2006; Morfeld *et al*., 2016; Schiffmann *et al*., 2018a). Deficiencies tend to be case specific and numbers of individuals affected are low. Due to the low growth rate and large body size of elephants, it is possible that nutritional inadequacies may go unnoticed for relatively long periods of time in a zoo setting.

Nutritional management in zoos

Within each zoo, captive elephant diets should be formulated in line with the zoo's dietary management programme using the skills of zoo nutritionists, curators, veterinary staff and keepers. The diet should be reviewed at least annually by appropriate staff, and proposed modifications raised. Forage consisting of grass, hay and browse should be the staple dietary component, comprising a minimum of 80% of the total dry matter (Ullrey *et al*., 1997). Nutritionally appropriate pellets should be fed according to the individual dietary needs, but in the range of no more than approximately 20% of the total dry matter. Exceeding this limit may lead to excess energy consumption. When feeding an elephant specific pellet, it is paramount to consider the recommendation provided by the manufacturer, assuming they have expertise in the species. Dietary items that deliver readily digestible energy, such as grains, bread, fruits, vegetables and low-fibre pellets should not be used in any significant quantity, although they may have uses for training purposes, the administration of medication, or geriatric animals. All food fed to the animal as part of a daily routine including items used for training, enrichment or public activities must be included in the daily diet ration calculations.

A review of the nutrient recommendations for both elephant species can be found in (Sach *et al*., 2019). Although species-specific differences may be present, evidence-based findings on corresponding requirements are lacking and further research is recommended (Bechert *et al*., 2019). Hence, based on the current knowledge, we consider our recommendations to be valid for both elephant species kept in European facilities.

Feed storage and preparation

As with all animal feed, appropriate storage conditions are essential to retain product quality, including appropriate insect and rodent control measures. All food storage should be designed in such a way to enable safe access by staff and limit wastage. A clear system for stock control and product traceability must be implemented.

Forage – must be protected from the weather (wet) and with good ventilation to prevent mould and degradation.

Browse- must be protected from weather (wet) to prevent mould/degradation and consumption from other pest species.

Pellets - purchased supplies should not exceed the amounts needed over a 4 to 6 month period in order to prevent degradation of vitamins, assuming ideal storage conditions. Most vitamins within pelleted feeds are stabilized for shelf life of up to 1 year however, products must be individually checked and an inventory with record of expiry date must be maintained for the animal feed stored.

Produce (vegetable and fruit) – must be kept under refrigeration

Feed items- Forages:

Hay and fresh forages

Grass hay is an ideal forage source for species adapted to eating plants high in fibre. It is important that the hay is of high *hygienic* quality, properly dried and cured. Hay should look green, and be free of weeds, insects, mould, twine, wire or any other foreign objects. Hay must be visually inspected before a delivery is accepted, and should be rejected if found to be substandard (mouldy, excessively dry and dusty, or off-colour). During the process of unloading a delivery, this testing should continue, and not only be applied to the first few bales or batches which may have been deliberately chosen by the merchant to give a good impression.

Given that elephants should have hay available at all times, and that obesity rather than energy deficiency is the primary concern, the hay used should be of a low *nutritional* quality (e.g., crude protein 5-8%, neutral detergent fibre 60-70%, acid detergent fibre 40-50 % in dry matter). Ideally, the grass should have been cut at a very late growth stage, with long, lignified stems. Hay typically used for production animals, cut at an earlier growth stage with soft, pliable stems and with a high proportion of grass leaves, is not ideal for elephants due to its high energy content. Because hay suitable for elephants is typically not produced for the hay market, and because farmers cannot sell the same amount of hay if cutting their fields as late as is reasonable for elephant consumption as compared to what they could sell cutting the same field several times, prospective contracting of farmers and fostering long-term relationships is recommended.

Considering differences in the dietary needs of individual elephants (e.g. breeding vs. non-breeding females), it is recommended to have various batches of hay with differing energy content/digestibility on site. Hay of particular grass species, such as reeds, has been used successfully by some elephant facilities.

For the use of fresh forage, the same principles apply (i.e. grass of late maturity stage with long, lignified stems). If at all possible, the use of fresh forage should receive priority over dried forage, but may be necessarily limited to the non-winter season. Reedgrass or elephant grass, or other tall grasses, may be suitable. In theory, using whole maize plants *without the cobs* would also represent a suitable elephant feed. When feeding fresh forages, their dry matter content needs to be accounted for in ration calculation.

If possible, access to grass paddocks can be provided to all elephants within the collection, although this may not be possible for some zoos due to space and weather limitations. Paddocks should have appropriate drainage, especially around high use areas, such as gateways and feeding stations to maximise the amount of time in which they may be used by animals. The time taken for elephants to consume small amounts of food via grazing is extremely important from a behavioural perspective and can assist in increasing the proportion of an elephants' time spent foraging. For facilities without copious grass paddocks, implementation of comprehensive feeding enrichment as a substitute is a must. The latter may present an opportunity to compensate for limited space as recently reported by Scott & LaDue (2019).

Browse

Browse is an essential dietary component, both nutritionally and from a behavioural perspective. It must be fed daily to all elephants throughout the year and may contain twigs, branches and stems or even entire tree logs. Consuming browse increases foraging time and has additional benefits for dental health.

A plan should be in place for adequate browse provision throughout the year, including the winter months when leafy material is not readily available. Browse can be preserved for other species by silaging, freezing or drying, but for elephants, due to the volumes required, this is mostly not feasible. Rather, stems and twigs without leaves should be provided on a daily basis, as well as evergreen species such as evergreen oak (*Quercus ilex*), bramble (*Rubus fruticosus*) or stinging nettle (*Urtica spp.*). Feeding conifers has proven successful for some collections.

Straw

Straw can be a suitable low-caloric fibre addition to the diet of elephants and can be mixed in with the hay ration to prolong foraging time, especially in high feeding nets. Due to the large amount of forage required by elephants, mixing of hay and straw generally appears to be the less feasible option as compared to the acquisition of long-stem grass hay of low nutritional (but high hygienic) quality. If mixing of hay and straw is done, the ratio should be determined in accordance with the dietary needs of the individual elephant. Like hay, straw must be of high hygienic quality, free of weeds, insects, mould, twine, wire or any other foreign objects and should be visually inspected before a delivery is accepted. Wheat or barley straw is preferred, as oat straw typically contains a higher energy content.

Lucerne

The elephants' requirements for bulky, low-energy roughage can be easily met with grass hay and straw, so that the more costly lucerne hay is typically not required. The feeding behaviour of elephants makes a loss of leafy material particularly likely when dealing with lucerne hay. Therefore, fresh lucerne or lucerne haylage would be considered more suitable due to the reduced leaf losses. Fresh lucerne or lucerne haylage might be used to increase the calorie and protein content of a specific animal's diet, under specific circumstances, such as with geriatric animals or animals of compromised health. However, providing a grass hay of higher nutritional quality most likely is a more feasible solution.

NOTE: if feeding of haylage is considered, it should be of excellent quality. Several elephants have died of Clostridial botulism due to feeding improper haylage!

For all forage items, gradual changes with a slow introduction of new material over the course of two weeks is recommended. The amount of the new diet item should be gradually increased over time so that after one week, it represents 50% of the forage portion, and is transitioned to the only forage after the second week.

Other feed items

Pellets

Except for special circumstances of particularly low forage quality or mishaps (e.g. sudden detection of forage spoilage due to roof leakage), there should be no need to provide elephants with pellets for maintenance energy requirements. However, a variety of pelleted feedstuffs is available. Some are manufactured specifically for elephants and are designed to be fed in very small quantities, with forage making up most of the diet (hay, grass, browse, straw). These pellets provide high levels of vitamins, minerals and protein, in a concentrated form so only a small amount is required to meet the elephants' nutritional needs. A combination of such a product with forages represents an easy and comparatively safe approach, because potential variation, especially in the mineral composition of the forages, is of little concern, given that the baseline is provided by the fortified pellet. With this approach, the individual provision of specific amounts to each individual, according to its body mass, is prerogative.

It is advised that pellet selection is made by the zoo's nutritionist or, if no nutritionist is on staff, by a nutrition advisor of the Elephant TAG or nutritional consultancy service, which is sometimes also provided by renowned manufacturers. To avoid digestive upsets, the introduction of any pellet into the diet should be gradual (increasing slowly over 2 weeks).

Fruits and vegetables (produce)

These should be fed in very limited amounts (less than 1 kg per elephant per day) and be documented as part of the daily diet ration. Produce is comparatively expensive, and amounts fed should not contribute to vitamin and mineral provision. Even small quantities of fruits higher in sugar may significantly contribute to energy levels in the diet, adding to the risk of obesity. High sugar fruits should be replaced with vegetables – ideally leafy greens. In appropriate quantities, their use in training may be valuable. As it is easy to condition animals to the use of high-sugar items, but difficult to then reverse the conditioning, it appears prudent to refrain from the use of such items from the very beginning and establish the use of leafy greens as training items.

Bread

Bread should be avoided. Should this be required for the administration of medication, use must be monitored.

Bran

Elephants can be reluctant to consume unfamiliar foods, therefore, it is appropriate to offer potential carriers for medication such as a bran mash periodically, so they will be consumed when needed. However, it should not be necessary to offer such items daily.

Vitamin and mineral supplements

The dietary concentrations of minerals and vitamins recommended for horses should in the most part be sufficient for elephants (Ullrey *et al*., 1997). Mineral deficiencies have rarely been reported and are best avoided through the adequate use of appropriate forages, supplemented with pelleted feed, rather than additional external supplementation where consumption is more challenging to ensure and monitor. In cases of determined or strongly suspected deficiencies (e.g. during pregnancy or lactation), commercially available readily formulated elephant supplements can be administered.

2.2.2 SPECIAL REQUIREMENTS

Staff behaviour

When changing the diet of elephants, it may be appropriate for staff to avoid eating those diet items (apples, bread) within the elephants' range of vision. In doing so, negative reactions by the elephants may be avoided.

Food analyses

Typically, it is recommended to analyse all feeds on a regular basis. However, the question each zoo must ask itself is: how will that information be used? Analysing feeds appears reasonable if there is a nutritionist on staff to make use of that information. Yet, even with a nutritionist on staff, or the use of a consultancy service, it may be a more cost-efficient approach to design a diet based on forages and a concentrated pelleted food that covers a range of possible nutrient values of the forages, rather than adapting the pelleted component each time a batch of forage is analysed.

Having stated the potentially limited use of nutrient analyses, there is no excuse not to perform hygienic assessments of all feeds delivered to the zoo. Even if there is no nutritionist on staff, or even if there is no dedicated commissary manager, it cannot be excused if there are no personnel trained in evaluating the hygienic quality of forages, vegetables and pellets. In particular for forages, given their relevance and bulk in herbivore diets, personnel dedicated to evaluating and either accepting or rejecting a delivery, and dedicated to proper storage and assessment of storage quality, is indispensable.

Presentation of food

Several studies indicate free living elephants of both species spend a considerable proportion (48-76.4%) of their day feeding, although, where feeding conditions are improved and food availability increased, elephants have been seen to reduce the total amount of time spent feeding (Beekman & Prins, 1989; Dougall & Sheldrick, 1964). There is debate surrounding the feeding pattern; several reports indicate that elephants feed almost continuously throughout a 24-hour period (Beekman & Prins, 1989; Laws, 1970). However, there is also evidence that elephants feed with distinct peaks (Sukumar, 1990). It is thought the feeding pattern may vary depending upon food availability, temperature (time spent in shade) and migration (usually to water).

It is commonly accepted that feeding in captivity must mimic the feeding behaviours of wild counterparts. A variety of complex feeding opportunities (e.g. at different height levels) to prolong foraging time throughout the day and night must be provided and provision for food delivery in evening/early morning must be made when personnel is typically absent. With respect to the temporal occurrence of major sleep periods, no extra food should be added between midnight and 6.00 am to avoid sleep disturbance with noise (Schiffmann *et al.*, 2018b), which is evidently not difficult to achieve. Keepers must periodically monitor this via night-time video recording of all animals, to ensure all animals are able to obtain access to food and ensure feeding events do not encourage anticipatory or stereotypical behaviours.

Diet monitoring

Appropriate monitoring of body condition and weight is essential and should be conducted at least four times per year. Visual body condition scoring has been demonstrated to be a practical and simple monitoring tool (Chusyd *et al*., 2019; Fernando *et al*., 2009; Schiffmann *et al*., 2018a) and is of particular importance if weighing is not feasible. Records must remain with the animal throughout its life and be recorded as appropriate (e.g. via ZIMS). Consequences of obesity in captive elephants are extremely serious and will affect the animals' long term captive health and welfare. There is strong evidence that obese animals are at increased risk of foot and joint lesions, altered metabolic markers and reduced reproductive success with increased labour length, dystocia, stillbirths and ultimately cow and calf death (Chusyd *et al*., 2018; Freeman *et al*., 2009; Norkaew *et al*., 2018; Olson, 2004).

Where animals are not achieving an optimum BCS, a documented plan must be in place to achieve the optimum BCS and with records kept of progress made. Daily, keepers must monitor diet consumption and report variations as appropriate. Individual diet plans must be made for each elephant and recorded.

2.2.3 VITAMIN AND MINERAL DEFICIENCIES IN CAPTIVITY

Vitamin E

Deficiency of vitamin E in herbivores causes a range of symptoms including necrotising myopathies, anaemia, reproductive failure, capture myopathy and white muscle disease (Dierenfeld & Dolensek, 1988). Elephants typically have low circulating plasma serum vitamin E levels compared to domestic herbivores (Papas *et al*., 1991), and differences have been noted between African and Asian elephants (Savage *et al*., 1999; Shrestha *et al*., 1998). Leaves may contain 20 times as much vitamin E as the stems of plants, but losses during haymaking can be as high as 90%, therefore zoo elephant diets based primarily on hay are almost certainly low in vitamin E. Consequently interest in the importance of supplemental vitamin E for elephants has resulted in supplementation products marketed specifically for elephants, which may be appropriate to use in specific circumstances, depending upon vitamin E provision in the overall diet (Kenny, 2001). While circulating vitamin E levels may fluctuate seasonally (depending on diet and potentially physiology), variation has not been systematically monitored in elephants.

Calcium

Elephants have their highest demands for calcium when tusk-growing (males) or lactating (females). Lactating females are estimated to need 60g of calcium per day and tusk-growing males require 8-9g/day (McCullagh, 1969). Calcium metabolism in elephants appears to be similar to equids with an absorption efficiency of approximately 60% from the diet, independent of dietary concentration, with excess absorbed calcium excreted in the urine (Clauss *et al*., 2003). In a study conducted in the UK, based on 14 collections, a minimum of 0,33-0,77% DM calcium was provided in the diet. This is in excess of the maintenance recommendation of 0,3% DM and suggests why deficiencies are rare in healthy adults at maintenance, but may be seen during parturition and lactation when demands are much greater (Partington, 2012). Calcium deficiency has been documented in individual cases during parturition*,* making dietary calcium supplementation recommendable prior to birth (van der Kolk *et al*., 2008). This could be achieved, for example, by additional provision of browse and lucerne hay in the month prior to birth (see EAZA Veterinary Guidelines for reproduction-related management in captive female elephants in Appendix II or at **EAZA Elephant TAG SharePoint**).

Zinc

A single case of zinc deficiency was reported in an Asian elephant causing a secondary immune deficiency, skin lesions which included superinfected vesiculobullae above the toenails and hyperkeratosis on the extensor surfaces of both elbows and on the tail. Dietary zinc level was adjusted from 21mg/kg to 53mg/kg in feed on a dry matter basis and significant improvements were seen within 2 weeks and lesions resolved after eight weeks (Schmidt, 1989). The recommendation for captive elephant dietary zinc concentration is 40mg/kg DM (Olson, 2004). Although in a study of 14 UK zoos, zinc levels in the diet ranged from 22-51 mg/kg DM, the values do not include the contrition of zinc from grass or browse in the diets, thus likely underestimating dietary status; however, no clinical signs of mineral imbalance were reported in that study (Partington, 2012).

2.2.4 Examples for daily ration quantities

Please note that the following daily rations serve as examples, making individual adaptation necessary before application. Ideally, a zoo should have a nutritionist on staff. If that is not the case, this task may fall to a veterinarian with some basic nutritional training or can be outsourced to a nutritional consultancy, of which there is a growing number in Europe, for a simple ration calculation. Alternatively, several manufacturers of zoo diets also provide nutritional consultancy. As with any business, the credibility of the service should be assessed by asking for references from other zoos and by plausibility checks. In particular, advice that appears to be tuned to use a maximum of pellets should be viewed with caution. Target overall diet composition (ingested roughage and non-roughage items) may be in the area of 10% crude protein, 60% neutral detergent fibre, and 40% acid detergent fibre in dry matter. Accurate calculation of the quantities required to cover the individual needs of an elephant would require constant analysis of the diet as well as monitoring roughage intake (by measuring offer and refusals) to allow estimation of the proportion of roughage and non-roughage diet items, which is impractical – all the more so if the recommendation of multiple feeding stations spread across the whole enclosure is heeded. Hence, continuous monitoring of an elephant's physical condition by weighing and body condition scoring is strongly recommended (Schiffmann *et al.*, 2019b). Subsequently, diet composition and quantities can be adapted accordingly.

A) Adult breeding female, body mass: 3 348kg, BCS: 5/10

Estimated daily dry matter intake [kg]: 3 348kg * 0,015 -> 50,22kg

Feed item	Recommended quantity	Proposals for food presentation
¾ Grass hay, ¼ legume hay	55-60kg	Topfeeder (e.g. hay nets), feeding holes
Straw	5kg	Topfeeder (e.g. hay nets), feeding holes
Browse, branches	Ad libitum	Topfeeding (e.g. bundles fixed on cables/winches)
Pellets (containing minerals & vitamins)	1-3kg	Puzzle feeder, scatter feeding, rewards during training sessions or management actions
Note: Quantity may be varied significantly due to reproductive status (lactating, pregnant) and corresponding needs.		

B) Adult breeding male, body mass: 5 278kg, BCS: 7/10

Estimated daily dry matter intake [kg]: 5 278kg * 0.01 -> 52,78kg

Feed item	Recommended quantity	Proposals for food presentation
Grass hay	45kg	Topfeeder (e.g. hay nets), feeding holes
Straw	30kg	Topfeeder (e.g. hay nets), feeding holes
Browse, branches	Ad libitum	Topfeeding (e.g. bundles fixed on cables/winches)
Pellets (containing minerals & vitamins)	2kg	Puzzle feeder, scatter feeding, rewards during training sessions or management actions

C) Geriatric (non-breeding) female, body mass: 2 934kg, BCS: 4/10

Estimated daily dry matter intake [kg]: 2 934kg * 0,015 -> 44,01kg

Feed item	Recommended quantity	Proposals for food presentation
½ grass hay, ½ legume hay	55kg	Topfeeder (e.g. hay nets), feeding holes
Straw	0-5kg	Topfeeder (e.g. hay nets), feeding holes
Browse, branches	Ad libitum	Topfeeding (e.g. bundles fixed on cables/winches)
Pellets (containing minerals & vitamins)	3kg	Puzzle feeder, scatter feeding, rewards during training sessions or management actions

D) Sub-adult male/female, body mass: 2 237kg, BCS: 8/10

Estimated daily dry matter intake [kg]: 2 237kg * 0,01 -> 22,37kg

Feed item	Recommended quantity	Proposals for food presentation
Grass hay	20kg	Topfeeder (e.g. hay nets), feeding holes
Straw	10kg	Topfeeder (e.g. hay nets), feeding holes
Browse, branches	Ad libitum	Topfeeding (e.g. bundles fixed on cables/winches)
Pellets (containing minerals & vitamins)	1kg	Puzzle feeder, scatter feeding, rewards during training sessions or management actions

***Calculations based on the following parameters:** maintenance requirement of daily dry matter intake 1-1.5% of an elephants body mass (Ullrey et al., 1997). Dry matter hay: 90% (Ullrey et al., 1997); recommended quantity pellets: elephant pellets, Kasper Fauna food: 1kg/1 000kg BM per day.*

2.3 SOCIAL STRUCTURE

Appropriate social grouping is key to successful elephant management and must be pre-eminent in all aspects of care.

2.3.1 BASIC SOCIAL STRUCTURE

Social Structure

The core social units of elephants are matriarchal, multi-generational family groups varying in size from two to 16, usually related, adult females and their offspring (Schulte, 2000). These groups are built on complex networks of social bonds that change in strength and quality over time depending on both relationships with other elephants and resource availability such as food and water (Archie *et al.*, 2006). This leads to fission-fusion societies with groups fragmenting into smaller units and re-joining according to conditions (Vance *et al.*,2008).

Social learning during development is crucial for the acquisition of normal behaviours (Lee & Moss, 1999). Young elephants learn from their interactions with other elephants which have a significant influence on their future behavioural, social, reproductive and maternal competence.

Bull elephants are not necessarily solitary (Lee *et al.*, 2011, Srinivasaiah *et al.*, 2019). In the wild, young males remain in the tightly bonded female society until they are between 10 to 20 years of age (Moss & Poole, 1983). Leaving the maternal group is a gradual process with them joining small, all-male groups within which they develop social bonds (Lee *et al.*, 2011). Young male elephants follow older musth males around learning reproductive behaviours (Rees, 2004). Bulls of all ages regularly interact with the female family groups and so have a diverse range of social relationships and extensive social choice (Lee *et al.*, 2011).

Social Structures in Zoos

Historically groups of elephants in zoos have been composed of unrelated adult females. This may limit opportunities for learning and displaying social, reproductive and maternal behaviours, which contributes to incompatibility and conflict between elephants and perpetuates poor reproductive success (Hartley & Stanley, 2016), and therefore raises welfare concerns (Clubb *et al.*, 2008).

Creating groups that better resemble the natural social structure and allow for this learning environment in zoos, is complex and challenging (Schulte, 2000). EAZA zoos have a long-term plan, created in cooperation with responsible EEP coordinators, that defines the purpose of their elephant herds and outlines the steps to be taken to achieve an appropriate group structure e.g. stable matriarchal family groups or bachelor groups wherever possible. This process can be enhanced through carefully considered transfers between collections, via the responsible EEP. For more information see chapter **2.4.7 Population Management**.

The zoo should maximise opportunities for every elephant to have unrestricted physical contact with other members of the herd for as many hours each day as possible (unless there are serious and justified reasons for such a separation). On the other hand, housing should be designed so that individuals can choose to be separated either physically or visually.

Female elephants must have social contact with other elephants at all times. If herds are kept, groups should contain at least four compatible females over 2 years old. Isolation and restraint during parturition have been associated with dystocia (Clubb *et al.*, 2009; Mason & Veasey, 2010; Hartley & Stanley, 2016). Cows that are unrestrained and were with the herd during parturition are less likely to show maternal aggression (Flugger *et al.*, 2001; Prahl, 2009). Hartley and Stanley (2016) found that a socially stable and compatible herd increased calf survival.

Bulls must be given the option to be in social contact with other elephants if they choose. Acceptable social situations for bulls include either housing bulls so they can have regular contact with the family herd, ideally with another bull present, in order to to facilitate social learning (i.e. 1 older bull, 1 younger), or housing a bachelor herd with other bulls of varying ages.

Sleeping together as a group has been demonstrated to be important for herd cohesion and individual relationships and a good indication of alliances and conflicts. Bulls may also sleep with the family group if given the opportunity (Williams *et al.*, 2015).

Keeping single elephants is highly not recommended and should be avoided. The only exceptions might be an old cow under exceptional circumstances where there might not be a better alternative. All these cases must be under review of responsible EEP coordinator.

2.3.2 CHANGING GROUP STRUCTURE

It is increasingly being recognised that relationships between individual animals change over time potentially making an individual incompatible with another individual or the whole herd. Identifying and responding to these circumstances are important for the animal's welfare. Adult females may become incompatible after the hierarchy changes in the herd which may be caused by introduction of new elephants, the birth of a calf to the cow under consideration or other herd members, or puberty in young females. In the wild family groups do separate, usually down maternal lines with a female and her offspring leaving the group. These animals may or may not come together again in the future. Incompatible females should be transferred with appropriate offspring, including adult female offspring where appropriate.

It is important to remember that an elephant that becomes incompatible with its existing herd may play an important stabilising role in another herd, bringing life experience and behavioural diversity with it. A transfer may be in the best interest of the individual and both herds. Some studies show that changing herd composition can have positive effects on social behaviour, whilst the stress associated with the move is neither prolonged nor severe (Dathe *et al.*, 1992; Schmid *et al.*, 2001; Laws *et al.*, 2007).

The role of older or geriatric animals which may have a broad and diverse experience should not be under-estimated. Even as non-breeding cows, these elephants can have an important matriarchal role in helping to stabilise a herd or teach younger animals and they should remain within the family groups throughout life (Dublin, 1983; Schulte, 2000).

Bull Management

Social interactions are very important for bulls of all ages. Adult breeding bulls will spend up to 60% of unmanaged time in a social situation (Wood, 2017). Bull housing and management should allow the bull access to the herd with the opportunity for him to separate himself if he chooses. Bulls and cows should have access to a shared all-weather outdoor space throughout the year. Zoos should develop facilities that allow the bull(s) access to the entire enclosure to allow for increased social contact with other elephants. Normal social development of both bulls and cows is essential to ensure social compatibility, appropriate reproductive behaviour and therefore successful mating. Physical and social contact with bulls has been suggested to reduce the likelihood of acyclicity and failure to mate in female elephants (Hartley & Stanley, 2016). Twenty-four hour a day contact between cows and bulls leads to increased pregnancy rates (Olson, 1994).

As in the wild, juvenile and adolescent bulls will increasingly spend time playing and socializing with other bulls rather than with the female group. Although much of this interaction will be with other adolescents, young bulls will seek social contact with the adult bull (often their father) which is important for social learning (Evans & Harris, 2008; Rees, 2004). This social interaction with other bulls is essential for their development and adolescent bulls should be given the opportunity to spend time with the breeding bull, or any other bulls (if held).

When a young bull reaches the age where he starts to exert pressure on young females within the group, consideration must be given to protecting the stability of the group structure. A good quality social network and behavioural understanding of the group is essential to ensure that good management decisions are made. If the young animal is deemed too young or not independent enough to leave the group, then increasing time away from the group with an adult or sub adult bull may serve as a transitional phase for the young bull and alleviate social pressure. As the young bull grows more independent, the interest in staying with the core group will lessen and his interest will be in being around other bull elephants.

Thus, zoo management must develop facilities to allow young males to remain close to the natal herd, experience bull social development and experience a gradual dispersal to a bull, recreating a fission-fusion situation through availability of multiple enclosures and housing spaces. Breeding collections have a responsibility to house their offspring for a considerable time (age +12 years), if they are to develop appropriate behaviours as adults.

Bachelor Groups

The purpose of the collection must be determined within the Long-Term Management Plan of the EEP and zoo.

- Appropriate bachelor group structure should be followed including having a 'guide' elephant, preferably a mature male (depending on previous social history) to act as a mentor/teacher/guide for young bulls.
- For best social benefit, bachelor groups should consist of more than two individuals and be developed to hold multiple compatible bulls. This allows for social behaviours between the groups. Bulls must be of varying ages for teaching/learning of younger bulls.
- The collection should have a plan in place for younger bulls challenging the role of dominant bull. If this happens, the original dominant bull may be housed separately or transferred to another facility or could be kept with the group in a subordinate role.
- The elephants should be given 24/7 access to each other (unless there are serious reasons preventing it), to allow elephants to naturally separate themselves using the enclosure space rather than creating physical barrier (closed gates) between bulls and allow elephants 24-hour access to inside and outside areas. This can benefit social structure by giving them the freedom of choice and not be confined to one area with the rest of the group.

- Monitor and record all major positive and negative social behaviours to allow management to make evidence-based decisions regarding the elephants' grouping and social structure, and safety of elephants and staff.

Group structure change

Any separations will have an impact on the confidence and social standing of individuals within the group. The continuing stability of the group should be a significant consideration during all animal management decisions.

Temporary separations
Temporary separations should be a routine part of the husbandry management of the animals with special consideration needed for juvenile members of the group. Calves form strong bonds and extended separation from the dam or other close family members can cause undue stress.

Prolonged separations
Prolonged separations of animals within the group should be avoided unless critical to the survival of the animal. If prolonged separation is required for incompatibility reasons alternative accommodation should be considered for the separated animal.

Introductions
When introducing new animals to a group careful consideration should be taken around the impact that a new individual can have on group structure. A good understanding of the social network and dynamics of the current group is important and the social history of a new animal will impact the success of any mixing within the group. Facilities should be in place to increase the chances of success with proposed mixes and putting the animals in fight or flight situations should be prevented. Success has been had using movable electric curtains to allow the animals to process information about each other before final introductions. This should be done in a pre-planned way, incorporating the personality of individuals into the planning process.

Integrating breeding bulls
Established, socially competent bulls should mix with cows that also have a good social understanding of bull behaviour and expectation. Caution should be taken if there is any doubt over capabilities of any member of the group. Olfactory provision of faeces for the group and bull can be given in advance of proposed introductions as it will serve to create expectation and initial introduction of change to all animals.

2.3.3 SHARING ENCLOSURE WITH OTHER SPECIES

Information can be found describing mixed exhibit examples using elephants. Like most species in captivity, there have been increased initiatives for mixed exhibits, some with mixed results.

Three general principles can be used as guides in developing mixed-species exhibits (Thomas and Maruska, 1996):

1. The difficulty of combining species is inversely proportional to the size of the exhibit (the larger the exhibit, the less chance of complications).

2. For educational reasons one should keep the geographical and biotope species consideration in mind.
3. The smaller the exhibit, the more critical the selection of the species (and numbers of) placed within it to ensure natural behaviour. This can be facilitated with better structured enclosures with lots of topography, rocky hills, trees, visual barriers (giving chances to escape) etc.

General considerations in any mixed exhibit initiative include:

- Safety for animals and keepers
- Management possibilities for keepers
- Enriching life for all concerned species
- Veterinary and nutritional management
- Educational value

Safety

When building mixed exhibits, thorough considerations need to be taken to accommodate safe surroundings for all species. As elephants would be the dominant species in the mix, one needs to be very creative with barriers to allow other species their "safe zone".

Principles that govern such safe zones can be broadly defined as:

- Developing "niches" for all concerned species in the same exhibit i.e."vertical distribution" where the need to share the same surroundings is not obligatory because the basic needs for the species are in separate parts of the exhibit (e.g. arboreal/terrestrial combined).
- Designing barriers as "escape" possibilities i.e "horizontal distribution" where species share the same surroundings, but elephants are not allowed to enter the whole outdoor area.

In both cases, indoor facilities need to be adjusted as well. Either by making them "other species (hoofstock, primate, hyrax, carnivore, bird etc.) proof" or making sure the elephants cannot reach the indoor areas of the other species. Another way might be the possibility to divide the outdoor enclosure into two parts for the night (for horizontal distribution).

Management possibilities

By having multiple species in the same exhibit, management might be very complicated. General principles of attention may be:

- In the discussion which species to choose, keep the management options as an important variable. For example, you would not want hands-on management needed for a smaller species if you have large mammals in the same exhibit.
- Make thorough risk assessments and have good discussions with the animal keeper staff to avoid any misunderstanding about agreed management possibilities.
- Be creative in your management possibilities and "think outside the box". Many species are flexible in the way they can be managed. One option which is used more often is "Recall" where animals learn to connect a specific sound to a certain behaviour (e.g. coming into the stable).

Enriching life for all concerned species

Mixed exhibits are chosen as enrichment for species and visitors, and one needs to take measure to ensure that their welfare is not compromised and that living in a mixed exhibit is beneficial for the welfare of each of the species. Issues to consider include:

- Spatial use of the exhibit by all species
- Possible conflicts between the species
- Separation of feeding places/ opportunities
- Separation of retreat areas, so escape runs do not end at the same point e.g. a stable entrance
- Choose complementing species
- Assess individual welfare by scientific behavioural studies

Veterinary and nutritional management

In addition to possible veterinary risks because of inter species dominance, one would need to consider inter species infectious diseases. Microbes can be pathogenic for multiple species. Some viruses can be lethal to one species, while it is relatively harmless in the normal host. Diseases to consider when mixing elephants with other species are tuberculosis, cowpox and Salmonellosis.

Some general points of consideration might be:

- Write a veterinary risk assessment protocol of potential disease transmission
- Adjust your preventive veterinary work according to the situation

One needs to consider how to avoid nutritional complications by species eating one another's food. For example, the "grazer/browser" complications. Creative solutions need to be considered to maintain proper nutritional management for each species.

Educational value

Considerations regarding the educational value of a mixed exhibit need to be discussed. Popular educational stories revolve around the habitat of the exhibited species (Kopje area, Serengeti plains etc.), and for this reason one might choose a geographically specific species (e.g. South African gemsbok).

Introduction of species

When starting a mixed species exhibit, considerations regarding a safe introduction of all species to the enclosure and to each other must be made. This may include:

- Let all species explore all parts of the enclosure without the presence of the other specie(s) and consider dominance issues when deciding which species enters first.
- Let all species get used to feeding sites and feeding routines.
- Do introductions carefully and under observation and have a plan B ready if there are complications during the introduction and the species must be separated again.

Examples of mixed exhibits using elephants

Elephants, being the largest land mammal on earth are on one hand challenging to design mixed exhibits for, but on the other hand easier because they are, without doubt, the "dominant" species in the exhibit. The safety, management and veterinary considerations are vital focus areas. It would be advised to have a thorough training programme (recall) for the elephants as part of the management procedure because in a Protected Contact setting this will be the way to deal with this species in a mixed exhibit. Exhibit wise, barriers especially, will need to be adjusted to accommodate the mix. While hoofstock species would be relatively easy to mix regarding barrier adjustments, primates would make it much more complicated.

A very good example is the African savannah exhibit in Boras Djurpark in Sweden. The herd without the bull is managed alongside African cape buffalo (*Syncerus caffer*), Grant zebra (*Equus quagga boehmi*), Sable antelope (*Hippotragus niger*), Blesbok (*Damaliscus pygargus phillipsi*), Common eland (*Taurotragus oryx*), Giraffe (*Giraffa* sp.), Ostrich (*Struthio camelus*), and Helmeted guineafowl (*Numida meleagris*). In Ouwehands Zoo, the African elephants are exhibited with red river hogs (*Potamochoerus porcus*) while in Thoiry and Peaugres Zoo they are mixed with warthogs *(Phacochoerus africanus*).

Asian elephants are also being kept more often in mixed exhibits, with many examples worldwide. For example, Asian elephants are successfully kept together with Chital (*Axis axis*) and Crab-eating macaques (*Macaca fascicularis*) in Dierenrijk Zoo and with Blackbucks (*Antilope cervicapra*) at Dublin Zoo. In Zurich Zoo, an all-male group of Blackbucks use whole outdoor exhibit space calmly in the presence of the elephants and retreat to their area when needed as their stable and a small outside exhibit are not accessible for the elephants. Although no real barrier exists, the blackbucks do not use the inside exhibits of elephants.

2.4 BREEDING

2.4.1 MATING

Anatomy and physiology

Male

The testes are located inside the abdominal cavity. They are tennis ball-sized structures just posterior to the kidneys. The elephant penis is muscular, like that of horses and humans. The length of the penis is approximately two metres, and is S-shaped. The other organs involved in the male elephant reproductive system are the seminal vesicles (sperm storage organs), the prostate gland, the ampullae and the bulbourethral glands.

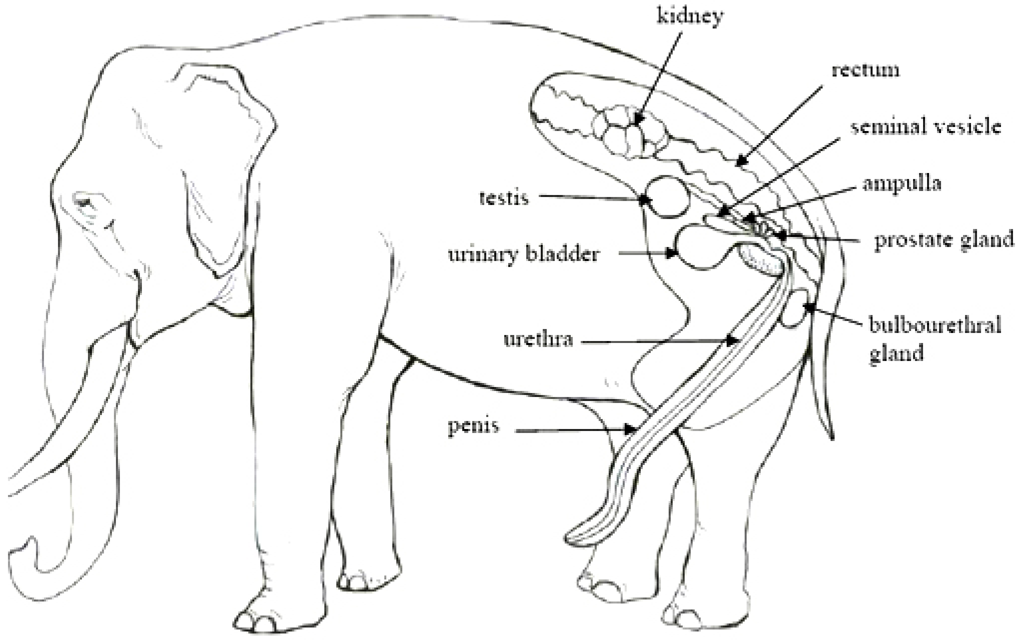


Figure 12 The male elephant reproductive system (modified from Hildebrandt *et al.*, 2006)

Female

Like a cow or horse, the female elephant has a bicornuate uterus, meaning there are two uterine horns. The ovaries are located behind the kidneys. The entire reproductive tract from the ovaries to the vulva opening measures up on average 2.5 meters. The lower genital canal is 68-88 centimetres long and consists of the vagina, vaginal opening and the vestibulum vagina. The vulva is positioned between the inguinal regions, ventrally between the hindlegs, which is different from most other mammals. The clitoris lies within the vulva opening and is about 40 centimetres long, about the same length as the vulva. The two mammary glands are located pectorally, between the forelegs. The placenta of the elephant is zonary and the gestation period of the elephant is about 20-22 months (Estes, 2012).

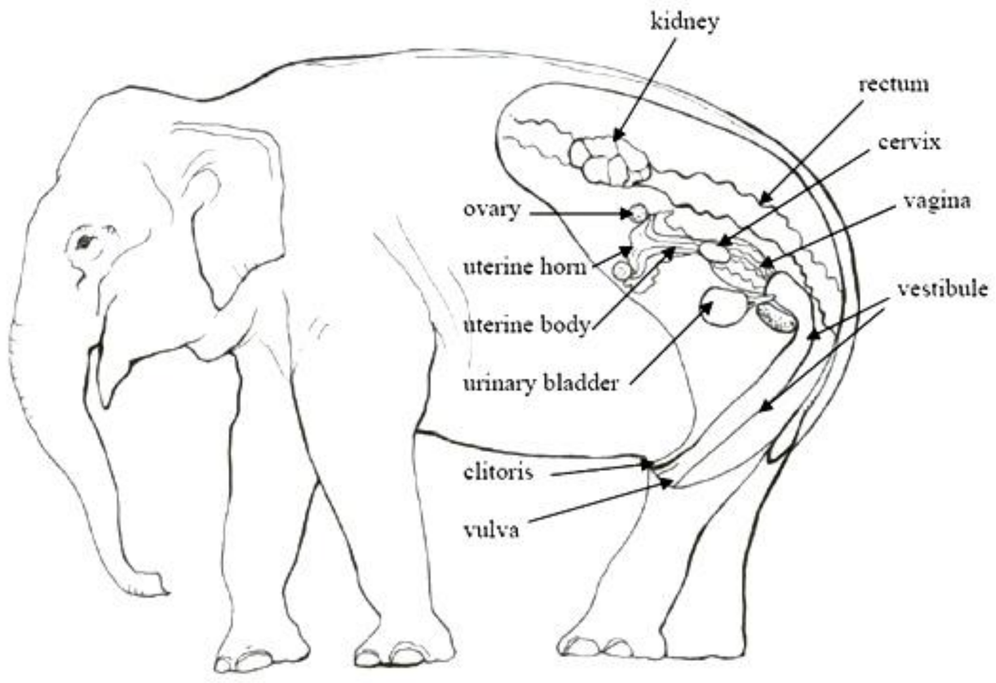


Figure 13 The female elephant reproductive system (modified from Hildebrandt *et al.*, 2006)

Basic endocrinology

Elephants have a similar reproductive endocrinology compared to other mammals. In both male and female mammals, GnRH (gonadotropin releasing hormone) is produced by the hypothalamus in the brain, which in turn stimulates the pituitary gland to release FSH (follicle stimulating hormone) and LH (luteinising hormone) into circulation. These two hormones stimulate the testes and ovaries of males and females, respectively, and this process can be influenced by pregnancy, lactation and contraceptive drug administration. Further details on the female elephant endocrine system are given in Fig. 14.

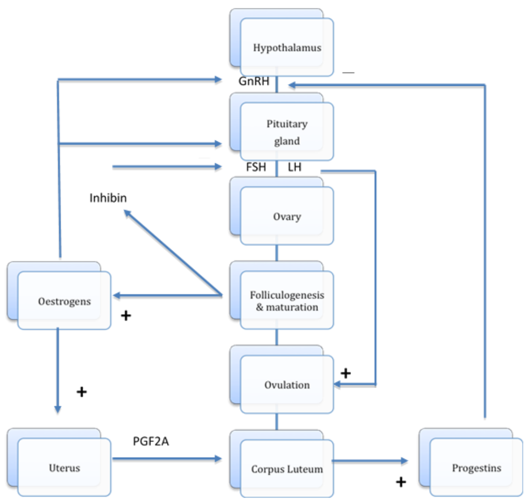


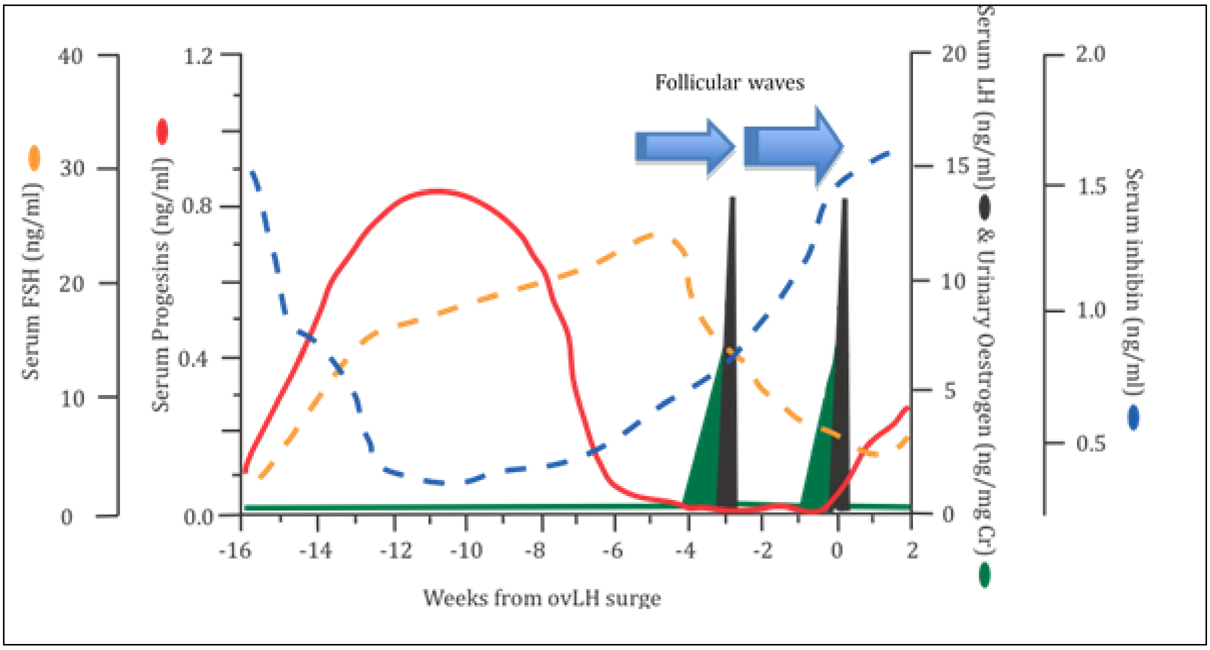
Figure 14 Schematic of the general female hormonal feedback system within the oestrus cycle

Analysis of circulating hormones and ultrasonography are both essential for the assessment of normal reproductive health and reproductive tract pathology in both the female and the male elephant (see Fig.12, 13; Hildebrandt *et al.*, 1998, 2011; Brown, 2000).

The oestrus cycle

Female elephants are spontaneous ovulators, polyestric (multiple cycles every year) and non-seasonal breeders (Lueders *et al*., 2012). Lueders *et al.* (2012) provide a well-referenced review of female elephant reproduction, including detailed ultra-sonographic images of the various physiological appearances of the female reproductive tract.

Unique to elephants is their exceptionally long cycle length of 13-17 weeks (see Fig. 15) and the double LH peak that occurs during the follicular phase (Brown, 2000; Czekala *et al.*, 2003; Brown *et al.*,2004). The first LH peak does not cause ovulation but leads to formation of multiple corpora lutea (CLs), with the second LH peak leading to ovulation of the single dominant follicle and formation of one additional CL (Lueders *et al.*, 2012). These LH peaks are usually 19-22 days apart (Brown, 2000; Czekala *et al.*, 2003). Only after ovulation, all CLs will produce progesterone metabolites (5α-progesterone, 5α-pregnane-3, 20-dione and 3α-hydroxy-5α-pregnane-20-one) (Heistermann *et al.*, 1997), which are only measurable with certain cross-species laboratory tests. If fertilisation has occurred, these CLs will remain hormonally active throughout the gestation (Lueders *et al.*, 2012).



Oestrus cycle	13-17 weeks
Follicular phase	4-6 weeks
Luteal phase	6-12 weeks

Figure 15 Model of the hormone secretion during the elephant ovarian cycle

It is essential to monitor female elephant cycling activity. This can be done via blood, urine, saliva, faeces and behavioural observations (Fiess *et al.*, 1999; Illera *et al*., 2014). Exposing the breeding bull to daily urine samples of the cow during the expected period of oestrus (Thitaram *et al*., 2009) can indicate the presence of pre-ovulatory pheromones through behavioural displays of interest (Rasmussen, 2002). For detailed description on how to monitor cycles, see document "To breed or not to breed" (Oerke, 2019), Appendix I or the **EAZA Elephant TAG SharePoint** page.

Female infertility

Due to good nutritional state in captivity, young female elephants can start cycling from as early as 3,5 years old (Hildebrandt *et al.*, 2011). If repeated oestrus cycles in the absence of pregnancy occur, reproductive pathologies may more likely develop, which may lead in turn to infertility in young cows (Penfold *et al*. 2014; Hermes *et al.*, 2004).

Infertility can also be caused by problems with regular cyclicity, e.g. having irregular oestrous cycles or the complete absence of oestrous cycles (flat liners) (Brown *et al.*, 2004). Temporary or permanent absence of oestrus cycles have been reported in captive Asian and African elephants of all age groups (Brown, 2000) and can be associated with seasonality, social status, hormonal dysbalances or reproductive tract pathologies (Lueders *et al.*, 2012).

Post-partum period

After giving birth, elephant cows experience a period with no ovarian cycles, known as post-partum anestrus or lactation anestrus. This is due to the suckling stimulus from the calf, which induces the secretion of the hormone prolactin from the pituitary gland. Accordingly, the presence of a suckling calf determines the length of the period until the first ovulation after parturition occurs again.
For the female elephant, the first ovulation after birth, or post-partum ovulation, indicates the first possibility for a new conception. In a study on European elephants, post-partum ovulation occurred on average after 40 weeks in Asian elephants (range 7 - 17 weeks) and after 49 weeks in African elephant females (range 11 -111 weeks). However, if females give birth to a dead calf or if the calf dies during or shortly after birth or is hand-reared and therefore is not suckling, the post-partum ovulation occurs significantly earlier. A summary of the data is given in Table 6 below:

	Calf alive and suckling	Calf dead or hand-reared
Asian elephant	50 weeks	14 weeks
African elephant	55 weeks	36 weeks

Table 6 Time between birth and post-partum ovulation (from Oerke & Heistermann, 2016)

This data demonstrates that inter-birth-intervals in captive elephants can be much shorter than the 4 year inter-birth-interval given for free ranging elephants if females conceive again immediately at the onset of post-partum ovulation. Depending on the breeding management and breeding recommendation, the post-partum estrus period can be used to have the cow conceive again or else skipped in cases where breeding must be postponed. Monitoring of reproductive status in the cow and observation of reproductive behaviour of the bull is important to detect post-partum ovulation and to time the next conception accordingly.

Male physiology

Mammalian male characteristics (anatomical and behavioural) and the production of semen (spermatogenesis, activity of male accessory glands) are under the influence of testosterone. Production of this hormone is regulated by FSH and LH release from the pituitary gland, and in elephants, this is not only dependent on age but also on social rank (Lincoln & Ratnasooriya, 1996). Musth is the temporary state of heightened sexual activity in the mature bull elephant and can last from a few weeks to several months, with temporal gland secretion and antagonistic behaviour as the main characteristics (Duer *et al.*, 2016). Increased androgen secretion, especially testosterone, is seen during this time (Brown, 2006). Assessment of musth can be made either through detection of the significant LH surge prior to the testosterone increase (Kaewmanee *et al.*, 2011), or non-invasively through measurement of the heightened testosterone levels in the urine and faeces (Yon *et al*., 2010; Ghosal *et al*., 2013).

Male sub and infertility

The fertility of a bull is dependent on maturity (age), bull behaviour and sperm quality. Testicular development and activity are dependent on testosterone levels, and only after pre-pubertal testes have grown to a diameter of more than 10 cm will viable sperm be produced (Hildebrandt *et al.*, 2000). Adult subordinate bulls may show reduced testicular size. Bulls that have been housed with the same cows from an early age can develop a sexual disinterest in their herd mates, even after reaching an age where sexual maturity is expected. On the other hand, when larger bulls are not present, the first musth can occur much earlier and male success can depend on the composition and comparative size of available females.

The initial part of the ejaculate contains spermatozoa that have been stored in the ampullae. If the bull has not ejaculated recently, the ejaculate will contain a high number of dead or dying sperm (Olson, 2004). This highlights the need for multiple matings in natural breeding situations, and for obtaining several fresh ejaculates if assessing fertility microscopically and/or collecting sperm for artificial insemination. Sperm quality is also reduced in aged bulls (Imrat *et al*., 2014). In cases where chemical contraception has been used for two to four continuous years, testicular development will be permanently affected, leading to male infertility at a later age, even despite cessation of contraceptive injections (Lueders *et al*., 2017). Where natural breeding is practised but does not result in pregnancies, assessment of male fertility including sperm quality is essential.

Conception

As already stated, availability of energy and body condition are the main factors responsible for the time of sexual maturity. The age of puberty is lowered by good housing conditions with constantly available food of sufficient quality but may be delayed under physiological and social stress. Asian elephants living in their country of origin, in captivity, show a reduced age of puberty. According to the studbook of Myanmar timber elephants, sexual maturity is possible between 5 and 8 years of age (Mar, 2002) and in Pinnawala, Sri Lanka, cyclic activity was detected by progestin measurements in blood in females as young as 5 years of age (Mendis *et al*., 2017).

In European Zoos, young elephant cows are able to show ovarian cycles at even earlier stage. It has been demonstrated via urinary hormone analysis that Asian elephant females show ovarian cycles from an age of 4 years onwards, whereas African elephant females start somewhat later, from an age of 7 years (Oerke, 2004). This data is supported by records of first births in both elephant species in Europe. Asian elephant females had their first births as early as 5 to 6 years of age, indicating conception at only 3 to 4 years. African elephant females produced their first offspring at 8 to 9 years which means that they conceived at an age of only 6 to 7 years of age. An overview of the youngest females that reproduced in Europe is given in Table 7.

As indicated in the Table, the first offspring of many of these young females was sired by their own father, or in his absence, by their half-brother. This demonstrates that zoos were obviously not aware of the fact that females are able to conceive at such a young age. In order to prevent inbreeding in both elephant populations in Europe, zoos that are keeping the bull with the female herd on a regular schedule must be prepared to either separate or exchange him. Asian elephant males must be switched before daughters reach an age of 4 years and African elephant bulls before daughters are 7 years old.

Asian: name	**born**	**zoo**	**1st birth**	**age at 1st birth**	**age at conception**
Wered*	1991	Ramat Gan	1996	5	3
Cinta*	2005	Tierpark Berlin	2010	5	3
Kaveri	1984	Paris	1990	6	4
Homaline*	1995	La Palmyre	2001	6	4
Saphira*	2010	Hannover	2016	6	4
* mated by father.				** mated by half-brother.	
African: name	**born**	**zoo**	**1st birth**	**age at 1st birth**	**age at conception**
Yoki*	1990	Ramat Gan	1998	8	6
Panya	2007	Halle	2016	9	7
Shara**	1978	Ramat Gan	1987	9	7
Lara**	1977	Ramat Gan	1987	10	8
Tammi	1987	Howletts	1997	10	8

all data: European Elephant Group, 2020 © A.-K. Oerke, 2020

Table 7 Age of youngest elephant <u>females</u> at time of first birth in Europe

In contrast to the onset of ovarian cycles in free ranging elephants, the first ovulation in captive females usually results in conception if a fertile male is around (Oerke, unpublished).

Another scenario is evident for elephant bulls. Table 8 shows the age of the youngest males that reproduced in Europe. This data proves that Asian elephant bulls are capable to sire offspring in a zoo setting when they are just 6 to 7 years old. African elephant bulls start somewhat later at 9 to 10 years of age. It must be noted that in all cases recorded here the males were housed alone with fertile females, so, in the absence of another, older bull.

Asian: name	born	Zoo	birth 1st offspring	age at birth of 1st offspring	age at successful mating
Dubas	1960	Almaty	1968	8	6
Bacho	1999	Tiflis	2007	8	6
Winner	2002	Izmir	2011	9	7
Boy	1922	Munich	1932	10	8
Emmett	1991	Whipsnade	2002	11	9
African: name	**born**	**Zoo**	**birth 1st offspring**	**age at birth of 1st offspring**	**age at successful mating**
Pambo	1992	Wien	2003	11	9
Vauka	1953	Kronberg	1965	12	10
Abu	2001	Halle	2013	12	10
Yossi	1974	Ramat Gan	1987	13	11
Tusker	1992	Wuppertal	2005	13	11

all data: European Elephant Group, 2020

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Table 8 Age of youngest elephant males at time of birth of their first offspring in Europe

Due to the increasing number of bachelor groups for both elephant species in Europe, young bulls get the chance to be with their peers and experience adolescence and puberty in a manner comparable to the wild. So far, most bulls being transferred from bachelor groups back into family herds proved to show good social behaviour and became experienced breeders. In contrast to the wild, breeding ability in captivity is not linked to musth. Even though musth occurs in some, but not all males in captivity, the phenomenon itself, the possible difference between Asian and African elephants, its relation to female reproductive status and mating activity of the bull is still not fully understood.

Natural breeding

Courtship behaviour observed in captivity is similar to that which is described in the wild precopulatory behaviour including; olfactory cues, flehmen in the male, trunk wrestling, driving, neck biting and attempted mounts. For copulation to be successful, it is essential that the female remains stationary during the mount. Intromission is then rapidly achieved, lasting from 8-45 seconds. Matings take place several times a day around the second LH peak of the female. Pregnancy rates can be enhanced by leaving the bull with the cow throughout the oestrus period (Taylor & Poole, 1998), as with multiple matings, the sperm quality will improve (Imrat *et al*., 2014).

Assisted reproduction (Artificial Insemination, AI)

Artificial insemination (AI) is a technique, which can be used under specific circumstances. AI must only be conducted with full approval of the relevant EAZA EEP. AI is a specialized technique that requires appropriate veterinary support and equipment. For more information see Hermes *et al*., 2007; Thitaram *et al*., 2009; Lueders *et al*., 2017.

2.4.2 PREGNANCY

Pregnancy

The presence of progesterone and its metabolites in urine and feces will rise during pregnancy. Prolactin increases markedly after five to seven months and can be used for pregnancy diagnosis from approximately 20 weeks on. However, prolactin is not 100% reliable, as it can also increases during pseudopregnancy (Lueders *et al.*, 2019). Transrectal ultrasound examination between 8 and 10 weeks in a standing elephant (or between 10 and 20 weeks in an elephant in lateral recumbency) is an important tool for pregnancy diagnosis (see Appendix II EAZA Veterinary Guidelines for reproduction-related management in captive female elephants). Ultrasonography can be a useful monitoring tool for assessment of foetal development where appropriate (Drews *et al.*, 2008; Brown, 2006).

Impending birth

It is useful to be able to predict the expected date of parturition (birth) in case assistance is required with the birthing process. As pregnancy length in elephants can vary between 625-690 days from the day of the last mating, even with the same dam a combination of observations and endocrinological measurements will be needed to predict the parturition date (Hildebrandt *et al.*, 2006).

Physical and behavioural changes

In the later stages of pregnancy, there can be a distinct development of the mammary glands and milk production may occur. Swelling of the ventral abdomen and around the vulva may be seen. As the calf moves into the birth canal, faecal ball size may reduce, and urination will occur more frequently and in smaller volumes. The mucoid seal of the cervix (mucus plug) will drop from the vulva.

Behaviourally, the cow may show restlessness, reduced appetite, evidence of discomfort, translated in pacing, separation from the herd, beating the vulva with the tail and climbing the cables of the enclosure (Hermes *et al*., 2007).

Hormonal changes

Impending birth can be indicated by changes in blood hormones, particularly serum relaxin which shows a sharp rise just before parturition (Brown, 2006). Measuring blood progesterone levels is one of the most reliable and objective methods of predicting impending parturition (Hermes *et al*., 2008).

Trans-rectal ultrasonography can supplement hormonal analysis for parturition prediction where appropriate. By monitoring the cervix and vagina for disappearance of the cervical mucus plug, relaxation of the cervix, and dilation of the cervix caused by the progression of the allantois sac into the cervix, progression of events can all be observed. When the latter happens, parturition is likely to occur within 12 hours.

2.4.3 Contraception

The influence of contraception on individual physiology and behaviour is still to be studied. For more information see "**GnRh-vaccine position paper**" by Lueders and Oerke (2016).
Contraception application should only be considered after careful consultation with the EEP coordinator.

2.4.4 Birth – see also the EAZA Veterinary Guidelines for reproduction-related management in captive female elephants (Appendix II)

Labour and birth

Once labour begins, the cow becomes restless and attempts to change position, sidekicks, lies down and stands up, and generally alters from normal behaviour (Hermes *et al*., 2008).

A bulge under the tail, initially containing the fluid filled amniotic sac, will appear and this will steadily become larger. As labour progresses, the chorioallantoic sac may rupture, leading to the discharge of a large volume of fluid from the vulva. The amniotic sac, which contains more mucoid contents, will be apparent as a bulge under the tail. This sac will rupture at a later stage, acting as a natural lubricant and protecting the calf against pressure from the surrounding tissues. In nulliparous cows (those that have not previously given birth), the calf will slide back and forth in the pelvic canal until the hymen ruptures. Eventually the calf will pass the caudal rim of the pelvis and with the aid of gravity slide down the vestibule to pass out between the hind limbs of the cow.

Posterior presentation is the most common (Schmitt & Mar, 1996; Hermes *et al*., 2008). The actual expulsion of the calf can be rapid and be completed within a few minutes (Hermes *et al*., 2008). The membranes of the amniotic sac can still cover the calf at this point requiring the dam to remove them (Schaftenaar and Hildebrandt, 2018). This may appear aggressive, with the mother rolling or kicking the calf and pushing it with her head. These are natural behaviours to stimulate the calf's breathing. In an inexperienced elephant, especially one that has not grown up in a breeding family herd, care should be taken that this behaviour is not overzealous, as infanticide has resulted in such situations. The matriarch and other experienced cows may separate an inexperienced dam from the calf until the dam is less excited, as has been observed in the wild.

Parturition will normally conclude within one to two hours after rupture of the allantoic sac (Dastig, 2001). If the amniotic sac is intact, there can be an uncomplicated delay in parturition with delivery of a healthy calf (refer to *Delayed parturition and dystocias* for overview of possible complications).

Nursing (suckling) is important to stimulate the oxytocin reflex of the dam, leading to further milk let-down and uterine contractions, which facilitates the passing of the placenta. The sooner the calf is nursing, the swifter the placenta will pass which, in the majority of the cases, occurs between 5-10 hours post-partum. Placental tissues are used extensively in elephant research, especially with regards to EEHV, and it is advised to contact EEP vet advisors prior to the expected birth to ascertain best storage practices of placental tissue samples.

Although the development of the bond between mother and calf should be prioritised, where possible, the umbilicus should be dipped in dilute iodine product and the calf should be weighed. Birth weights in captive elephants in Asia vary between 54-70kg (Dastig, 2001) compared to 106 -120 kg in western countries. Meconium (first faeces) is passed anywhere from four minutes to three hours after birth.

Elephant calves stand up quickly but may struggle with the onset of nursing (suckling), and some assistance might be required. If nursing is unsuccessful, both the calf (suck reflex) and the dam (mastitis, no milk, etc.) need to be examined. Umbilical hernias can also occur with some frequency but usually heal over as the calf grows.

Delayed parturition and dystocia

Delayed parturition once labour has started or obstructed labour (dystocia) can occur in both nulliparous and multiparous dams and can lead to the death of the calf and sometimes the dam.

There may also be a correlation between long gestations (or interrupted parturition) and the likelihood of stillbirths (Kurt and Mar, 1996). Typically, gestation is shorter for twins (Schmitt & Mar, 1996), and the second calf can be born up to three months later than the first, especially if still-born (Hermes *et al*., 2008).

Possible causes of delayed parturition and/or dystocia include:

- Age at first birth: Most frequently, dystocia is seen in primiparous females over 20 years of age (Hermes *et al*., 2008). This may be related to an increase in pathological conditions of the reproductive tract associated with uninterrupted, repeated oestrus cycles (Hermes *et al.*, 2004) and contributes to higher rates of stillbirths from older primiparous dams. Several anecdotal reports describe the suffocation of the calf in the vertical part of the birth canal in older, primiparous elephants due to the non-elastic perineal skin and vulva in these elephants.
- Maternal obesity: Several studies have shown a positive correlation between maternal weight at birth and calf weight, and between calf weight, prolonged gestation and stillbirth (Clubb & Mason, 2002). Maternal obesity can cause narrowing of the birth canal from fat tissue deposits in domestic animals.
- Low blood calcium: Hypocalcaemia of the dam leading to delayed parturition has been described in captive elephants (van der Kolk *et al*., 2008), as there will be inadequate calcium in the blood to facilitate uterine muscle contractions. Calcium rich diets during pregnancy are therefore advocated. See chapter 2.2 **Feeding** and Veterinary Guidelines for reproduction-related management in captive female elephants in Appendix II.
- Malposition of calf: Although rare, it has been suggested that anterior presentations can lead to dystocia more often than posterior presentations.
- Malformations: Congenital deformities and malformations caused by intra-uterine infections can all lead to dystocia, as is the case in domestic animals.
- Foetal death: Systemic infections that can affect the foetus include salmonellosis and cowpox virus.
- Scars from a previous vaginal vestibulotomy might lead to dystocia, but no cases have been reported yet.

Dystocia intervention

Dystocia is hard to recognise based on behavioural changes only. Correct interpretation of the progression of the parturition, or lack thereof, requires ultrasonography (Schaftenaar and Hildebrandt, 2018). Ultrasonography is a procedure that all adult elephants can be trained to undergo.

In the case of delayed parturition, calcium levels might need to be corrected and physical intervention may consist of transrectal massage of the pelvic roof and side walls, as well as of the vagina, in order to stimulate passage of the calf and initiate uterine contractions (Ferguson reflex). The administration of oxytocin to stimulate uterine contractions may also be required but the cervix must be fully dilated. If oxytocin is administered and the cervix is not dilated sufficiently then there is a significant risk of rupturing the uterus. In these cases, veterinary advice must be sought; ultrasound scanning is the only way to ascertain the dilation of the cervix.

In the case of dystocia with foetal parts ultrasonographically visible in the birth canal, surgical intervention may be possible by performing a vaginal vestibulotomy (incision of the wall of the vaginal vestibulum, to gain access to the birth canal). If the calf has died and cannot be extracted otherwise a foetotomy (dissecting the calf within the dam and removing it piecemeal) may be necessary (Schaftenaar, 2013).

Parturition protocol and preparation

A birthing plan must be drawn up well in advance of parturition and detailed records must be kept on the progression of the pregnancy, parturition, and post-natal period, concerning both the dam and the calf. This plan should encompass all possible scenarios (during both day and night) and mitigation strategies, consideration of human safety, and the health and wellbeing of the dam, calf and herd in general. Essential elements of a parturition protocol, inclusive of veterinary input, are:

- Contact details for all staff involved, including veterinarians and reproductive specialists.
- Mating/insemination date, and length of previous gestations (if applicable, as the mean length will give an expected due date). Parturition window is one month either side of the expected due date.
- Preparations required include alternations to enclosures, parturition area, hand-rearing policy and formula if needed.
- Agreed monitoring techniques for parturition prediction (ultrasonography, blood progesterone product measurements, behavioural observations, physical changes), timings and frequency, as well as interpretation of test results and details of laboratories used.
- Agreed monitoring during parturition (blood calcium levels, ultrasonography, and behavioural observations), timings and frequency as well as interpretation of test results and intervention during delayed parturition.
- Intervention in case of dystocia including point of first examination, decision making based on various scenarios, intervention methods, and emergency drug dosages (dam and calf).
- Staff capacity.
- Equipment and site preparation.
- Dam management during parturition.
- Calf management after birth.
- Dam reproductive and general health (as well as that of the herd).
- Research samples, packing and destination.

The birthing plan should be an evolving document that builds on the experience of each birth and the experience of other collections. It should be practically applicable and reflect current knowledge.

It is strongly recommended that collections with pregnant females and imminent births contact the EAZA Elephant TAG, review the Veterinary Guidelines for reproductive management guidelines (Schaftenaar and Hildebrandt 2018), as well as papers detailing obstetrics in elephants (Hermes *et al.,* 2008). The EAZA Elephant TAG have collected video footage of births and a DVD is available to review prior to birth. For more information, please contact EEP coordinators.

Enclosures

All enclosures where the dam and calf are likely to be kept must be calf-proofed well before the expected due date. All enclosure fixtures (permanent and temporary/enrichment) must be assessed prior to birth for suitability and safety of the calf.

Inside enclosure:

Temperature control: It must be possible to have an area where the temperature may be increased for new-born calves or if individuals are unwell.

Substrate: The substrate in the calving area needs to be considered. A sanded floor or pasture will provide better grip for the dam during labour and for the calf post-partum. A variety of substrates might be provided to allow the calf to develop and explore.

Feeders and water: These features must be at appropriate height for a calf to reach. If a pool is present, it should be checked so as to be safe for the calf.

Boundaries: All boundaries must be assessed, and calf-proofed prior to birth. Calves should not be able to access areas that could endanger themselves, the keepers or visitors. There must be no possibility that a calf could become stuck in bars, electric fences or small gaps etc. It must be noted that the voltage of electric fences for adult animals may be harmful to new-born calves and thus an assessment should be made of appropriate mitigation prior to birth.

Training and treatment facilities: Facilities should be designed and installed before the calf is born and a training plan should be put in place.

Outside enclosure:

Boundaries: must be calf proof as per above.

Shelter: must be provided to protect the calf in adverse weather and from extremes of heat and cold.

Pool: may be provided but must be safe for calf to enter and leave.

Mud wallows: care should be taken that wallows are not too adhesive or deep to prevent a calf from becoming stuck.

Management during birth

A cow with the following criteria does not require restraint during parturition:

- An apparently healthy gestation
- Is in an appropriate social environment
- Has birthing experience (hers or others in the group)

Chaining must only be considered where a herd birth is <u>absolutely not achievable</u>. This may be justified with an inexperienced, primiparous dam that does not have other experienced cows within its herd or with multiparous cows that have previously shown significant aggression towards their calves. Staff and elephants should be familiar with the process of chaining, all required facilities should be present (i.e. eyelets, floor-bolted rings) and at no time should human safety be risked during the birthing process. Where necessary, the calf should be removed, dried, examined, helped on to its feet and introduced to its dam as swiftly as possible, to ensure appropriate bonding.

2.4.5 Development and care of young

For Asian elephants within the EEHV risk period (one to eight years old), it is essential to thoroughly review the EEHV section, see "European recommendations for monitoring exposure to Elephant Endotheliotropic Herpes Virus (EEHV) in young elephants." in Appendix III.

It is essential that calves be exposed to herd life in their early years, to enable appropriate social development and learning so that they may go onto be socially and behaviourally competent adult elephants, in line with the mission of the EAZA Elephant TAG.

Opportunities must be given for young male calves to choose to move to the periphery of social groups in a fission-fusion social system. They will spend increasing amounts of time with other male calves and the breeding bull but should still have the opportunity to spend time (until appropriate) with the female herd.

Female calves must remain with the natal herd and should not be separated from their mother. When appropriate, sub-groups of the matriline may be required to move to new facilities e.g. grandmother, mother, aunt and daughter, although this must be done in line with recommendations from the EEP and with the social well-being of all individuals in the centre.

Records

Records must be kept, ideally on ZIMS (or other recognised record management software), of experiences that calves are exposed to so that future collections will be able to use this information should it be necessary. Examples of experiences include births, deaths, mating events and introductions of any new animals.

Enrichment

The early years of learning for a calf are critical and a whole range of enrichment should be available to the calves to encourage them to explore. In well-established elephant families, the calf has the opportunity to play with other members of the herd which is enrichment in and of itself. The range of enrichment provided must be suitable for the calf, bearing in mind safety (smaller animals not becoming trapped) and size (manipulable by smaller trunks etc.).

Environment

Environment should be as complex as possible in order to encourage natural behaviours in the calf e.g. foraging, bathing (water, mud, dust), digging and general exploration of their environments. This will assist mental and physical development.

Assisted feeding

It is imperative that the calf remains with its mother (and family), as much as possible, to ensure any bond formed is not lost and continues to develop. In the case of absence of suckling, every effort should be made to encourage this and there are many cases of supplementary feeding that can occur alongside normal, but inefficient, suckling.

Colostrum should be given within the first 24 hours, preferably when the calf is <6-12 hours old, ideally with colostrum milked from the dam. If this is not possible, fresh or frozen elephant plasma can be administered orally within the first 24 hours. Artificial or horse colostrum can be used, or elephant plasma administered intravenously into the calf in a method similar to that for a foal. Bovine nursing bottles and nipples are suitable, with the nipple hole slightly enlarged to allow a steady drip when tipped. Calves must find a comfortable nursing position, typically with trunks elevated or propped on something (i.e. hanging canvas, hay bales). Sometimes covering the body up to the ears (face visible) with a sheet or blanket may provide additional comfort to the neonate.

While the dam's milk is always considered best (extracted by hand, using a breast pump, or with oxytocin to facilitate let-down (Emanuelson & Kinzley, 2002), several milk substitutes have been successfully used for elephants (Mainka *et al*., 1994). However, both carbohydrate fractions (Osthoff *et al*., 2008) as well as lipid components (both size of droplets and fatty acid composition (Osthoff *et al*. 2005, 2007), differ radically in analysed elephant milks compared with these species, so careful modification or supplementation may be required to minimise digestive imbalances and maximise utilization of these substitute milks. Furthermore, elephant milk contains high levels of glucosamine and specific immunoglobulins (Takatsu *et al*., 2017), which is likely not duplicated by any of the common replacement milks. Nonetheless, elephants have been reared on several formulae.

- Grober Company, Ontario, USA (www.grober.com) produces the most commonly used elephant milk replacer in North America and full instructions are provided with this. Two versions of Elephant Gro are available, one formulated for African and one for Asian Elephants. It is also recommended that a plan to reintroduce the calf to the dam should be drawn up. There are instances of successful reintroduction after a period of up to 10 days.

- SMA Gold (SMA Nutrition, Maidenhead, Berkshire, UK) was used by both Whipsnade and, more recently, Twycross zoos with success; (N. Dorman, pers. com.). The calves found it palatable, and gained sufficient weight, though care must be taken to ensure calves are getting adequate nutrition (as outlined above).

- Salvana (Salvana Tiernahrung GMPH, Elmshorn, Germany) milk formula was used to successfully hand rear a calf in Berlin Zoo (Ochs *et al.,* 2001). However, while this formula contained appropriate nutrient levels as determined from published data, ZSL Whipsnade Zoo found their calf did not drink enough of it to put on sufficient weight (Flach *et al.*, 2007) and Twycross found it appeared to cause a stomach upset (N. Dorman, pers. com.).

The most recent attempts with milk replacers (e.g. Ostrava Zoo, Leipzig Zoo) used mixture of Salvana and horse milk replacer. Its composition mimics the natural composition of elephant milk (mixture of 2/3 albumin "horse type" milk and 1/3 cassein "cow type" milk (Mainka *et al*., 1994). However, it is important to notice that the composition of milk (including fat) changes with time of rearing (Abbondanza *et al*., 2013).

Some caretakers of orphaned elephant calves add faeces to the milk replacer with the intention to establish a gut fauna in the intestines of the calf. There is no scientific evidence that this works. Elephants are hind gut fermenters, which means that the microbes added in the food have to pass the stomach, where they will be killed by the low pH. Besides, providing random microorganisms by mixing them in the milk replacer may result in intestinal problems. Natural "transfaunation" is a process that begins during the birth. The vaginal microbiome is the first to colonize the new-born and will soon be followed by the gut microbiome of the mother and herd mates due to "natural contamination". Close contact with other elephants should provide sufficient opportunities for the calf to establish its own proper microbiome.

Young calves may only be able to consume 0.5 litre in a single feeding, fed initially on demand (Emanuelson & Kinzley, 2002), every two hours including night for very young calves. Although suckling bouts may be short in mother-reared calves (<2 min for African elephants), suckling takes place frequently. Some research suggests much of the nursing takes place at night (Andrews *et al*., 2005). Targeted milk intake for new-borns ranges between five and eight litres per day. Calves require between 600-800 kcal per 100kg BW. By three months of age, the schedule interval of feeding may be increased to three hours. Small amounts of solid food such as hay and other adult elephant feeds may be of interest to the calf as early as one or two months of age (Emanuelson & Kinzley, 2002), however, these are mainly the result of copying of adult behaviour than proper solid food intake.

Rietkerk *et al.* (1993) provide an analysis of Asian elephant milk taken monthly after the birth of a calf that demonstrated higher fat content than previously published data, although fat content was also variable and high (4 to 12%, wet basis) over 280 days of lactation, compared to another study (Mainka *et al*., 1994). A more recent and comprehensive longitudinal study (Abbondanza *et al*., 2013) of Asian elephant milk composition showed that fat content of milk continued to increase up to 30 months of lactation, while protein and carbohydrate contents remained stable or slightly decreased, thus providing a more energetically dense milk for the growing calf. This information suggests that differing milk compositions, corresponding to different life stages, have to be considered for optimal assisted feeding of elephant calves.

At eight months, Flach *et al.* (2007) introduced a feed of desiccated coconut, oatmeal, Horse and Pony pellets (Clarke & Butcher, Soham, UK), calcium carbonate and an equine vitamin/mineral supplement (Equivite Original, Spillers, Milton Keynes, UK). Hay and grass were available as for the adults. This calf was weaned by 18 months (with the milk intake reduced over six months) and was on an adult diet a few months later. Although calves nurse for up to three to five years in nature, they can certainly be successfully weaned with no apparent problems by two years of age; suckling until older, however (even if with just water) may be important for well-being of the individual.

Published growth rates for hand-reared calves vary between rapid growth of 1,0 – 1,2kg a day (Rietkerk *et al.*, 1993; Reuther, 1969) to slower rates of 0,7 kg/day (Flach *et al.*, 2007), averaging as much as 0,9 kg/day during the first year. Mother-reared calves in captivity vary from 0,4 kg/day (Andrews *et al.*, 2005) to 1,1kg/day (Rietkerk *et al.,* 1993). Optimal growth rates are not known, as there is no published data from the field. It is possible that if growth rates are not optimal, this may result in health issues. Therefore, more research is needed on growth rates and the effect of birth weight and captive diets on these rates.

Training

All calves should be trained by one year of age in PC system, to achieve the following:

- Examination of mouth, ears and eyes
- Weighing and, if available, measuring body temperature
- Foot care

- Intravenous access (blood collection, injection)
- Rectal access
- Acceptance of intramuscular injections
- Acceptance of ultrasound examination
- Trunk washes for disease screening and clinical monitoring e.g. tuberculosis, herpes virus

For more details on training see chapter **2.6.2 General handling**

2.4.6 HAND-REARING

Hand-rearing is not recommended and must be conducted with the full approval of the EEP coordinator only. If necessary, assisted feeding can be provided to ease the start of the calf and mother (for more details see previous chapter 2**.4.5 Development and care of young – Assisted feeding**).

2.4.7 POPULATION MANAGEMENT

The EAZA populations are managed by the Asian Elephant EEP and the African Elephant EEP. The EEPs are under the auspices of the EAZA Elephant TAG whose mission is as follows:

"African and Asian elephants are flagship species in our zoos; principally their role is in education and raising conservation awareness. The TAG seeks to establish self-sustaining and genetically diverse populations of elephants in EAZA zoos, promote excellence in all aspects of management and welfare of these iconic animals and develop the knowledge of elephant care professionals. The TAG will maximise the conservation support and research from zoos to elephant conservation."

In line with the spirit of EAZA, all participating zoos are partners, whether they hold a breeding herd, bachelor group or geriatric female group and cooperation with the EEPs and the TAG is essential.

Both EEP programs have Long-Term Management Plans (LTMP) describing the demographic and genetic population management strategy for the EEP population including strategies for research, husbandry developments, education and conservation activities, etc. The latest version of LTMPs for both species can be found on the **EAZA Elephant TAG SharePoint**.

Goals of population management in Europe are similar to other regional *ex situ* management breeding programmes, although the specific issues faced may be different. Close cooperation with other regions is essential in all matters (e.g. the exchange of bulls).

The sexual maturity and the successful breeding of the herd, as a consequence, is not only dependent on the age and physical health of the individual, but also on the structure of the social group and its experience regarding mating, births and the development of young. Therefore, the natural pattern of the movement of the different sexes in an elephant herd is mimicked by the way elephant transports are recommended in the modern elephant EEP management. In practical terms, it means that a female and her female offspring should never be separated and should be the base of family units (which include mothers and daughters with offspring).

Males and females are born in equal numbers, thereby presenting a challenge. Therefore, collections develop facilities which allow young males to stay within the natal collection (not necessarily a breeding herd) until a much later age (12+ years).

The understanding of the dynamics of bachelor groups is also helpful for our managed programs in order to deal with individual males that are in need for suitable placement. It is of the utmost importance that bachelor herd facilities for each species to be developed further; these facilities will act as a genetic reservoir but also as an opportunity for the development of necessary social behaviours between bulls. Bulls should be kept in a bachelor facility both when first leaving the natal collection, and subsequently when they are not needed in a breeding situation. The facility should not primarily function as a retired-male facility, but as a component of the genetic reservoir of the population.

As part of the EAZA Ex situ Programme (EEP) structure, there are various population management tools available that are important to maintain viable and healthy populations. This includes (temporarily) preventing breeding of certain individual animals through temporarily or permanent contraception (see chapter 2.4.3), keeping males and females separate to prevent mating during the period when the female is in oestrous, maintaining bachelor group for non-breeding animals and applying a breed and cull strategy (see for EAZA's culling statement: **https://www.eaza.net/assets/Uploads/Position-statements/EAZA-Culling-statement.pdf**). These tools each come with different pros and cons that need to be taken into consideration when an elephant holder is required to make decisions to avoid elephant population to grow beyond carrying capacity. It is important that elephant holders follow the breeding and non-breeding recommendations of the respective elephant EEPs and that they do not use any of the population management tools without prior consultation with the EEP coordinator.

2.5 WELFARE AND ENRICHMENT

Elephant social organisation and behaviour is complex. There is a great focus and move towards creating related, socially compatible herd structures. The proper social life of animals is essential and of higher value than any enrichment humans can provide. Nevertheless, there are some key points that should be remembered when considering captive elephant behaviour and welfare:

- Elephants have a large behavioural repertoire.
- Elephants are highly intelligent and demonstrate learning and problem solving, as well as social intelligence.
- Calf development through play and social exposure is essential to long-term behavioural well-being.
- Elephants can have large home ranges and roam over large distances (8 – 22km/day) in order to exploit resources.
- Elephants are generalist/opportunistic feeders evolved to be adaptive and flexible in order to cope with a variable/unpredictable environment and diet.
- Captive elephants generally seem to sleep in short bursts, totalling about three to four hours sleep per night only (Walsh, 2017). Thus, elephants continue to be active after the public and keeper staff have left (e.g. Horback *et al*., 2014; Walsh, 2017). Therefore, collections should work towards achieving a normal behavioural repertoire (including feeding) throughout the full 24-hour period.

Although we tend to think about behaviours which may indicate a negative welfare state, it should not be forgotten that there are also behaviours that can be used to indicate a positive welfare state. In particular, behaviours such as social play and social contact can be seen as indicators of positive welfare. Behavioural indicators of negative welfare include abnormal repetitive behaviours such as stereotypies and excessive anticipatory behaviour.

Expression of stereotypies in zoo elephants is highly variable between individuals. For example, a study of elephants in UK zoos recorded stereotypic rates over 24 hours of 0-60,8% of activity budget (Harris *et al.*, 2008 a,b). This likely reflects the complex histories of some animals (e.g. wild caught individuals, rescued individuals, complex health status, limited social experience) and emphasizes the importance of managing their welfare on an individual basis, ensuring calves are able to develop a 'normal' behavioural repertoire and grow up to be behaviourally competent adults.

It is important that zoos work to understand the causes of any stereotypies performed by their elephants, rather than just focus on stopping it. This is because stereotypies are complex and difficult to stop once they are part of an individual's established behaviours. There is a need to understand what caused the behaviours to initially develop, both to assist in avoiding their development in the future in other elephants within the facility (as this is likely easier to do than stop already present stereotypies) and to help ensure that any actions taken or changes made to reduce existing stereotypies do not inadvertently cause more stress to the individual.

Physiological indicators of welfare

Physiological indicators of welfare are based around hormones, particularly those associated with anxiety or stress. The most common are glucocorticoids, hormones produced by the adrenal cortex, which increase at times of stress or excitement. Glucocorticoids can be measured in blood, saliva, urine (to be collected at the same time of the day). In zoo animals, these hormones are most often measured from faeces (as these samples are easiest to obtain); however, this causes problems due to the time taken to pass through the body prior to excretion. A faecal glucocorticoid result can only provide a level for the previous approximately 24 hrs rather than for a specific point in time. Faecal glucocorticoid metabolites are what is actually measured, and these are usually a composite of levels present in the animal over a number of hours prior to sample collection. In addition, for most species, including elephants, a 'normal range' of glucocorticoids is not yet known. It is therefore challenging to know whether an elephant is exhibiting levels of glucocorticoids within normal range or indicating stress. It is important to consider that excitement can also trigger glucocorticoid production and therefore important to assess the environment and events around the elephant which may be causal factors.

Health as an indicator of welfare

Health parameters can also be indicators of welfare. For example, animals that are experiencing negative welfare can also experience compromised immune function and therefore be more susceptible to injuries and illness and display other indicators of poor health (e.g. irregular oestrus cycles, reduced muscle tone and definition, decreased flexibility in locomotion and movement, and failing to appear bright alert and responsive). Similarly, poor health can reduce welfare, for example where it causes pain or restricts the individual's ability to perform certain behaviours.

Good veterinary and health records are essential in allowing the identification of individuals who maybe prone to repeated instances of the same issue or to a high number of veterinary interventions. In cases such as these, it is important to review the elephant's entire situation (e.g. social and physical environment as well as exposure to disease and pathogens) in order to ensure that potential triggers are not missed.

Enrichment

Enclosures should be designed to provide a flexible, complex environment, which promotes cognitive challenges on a day-to-day basis and the opportunity to express a wide range of behaviours.

In addition to good enclosure design, environmental enrichment is an important tool for further providing captive elephants with stimulation and encouraging them to exhibit a wider repertoire of behaviour. Enrichment should occur throughout the elephant enclosure (indoors and outdoors) and be part of the daily routine. Enrichment should be goal based, recorded and evaluated.

Enrichment should be viewed as one husbandry principle to be used in a holistic husbandry portfolio to cultivate positive welfare. It is not the only solution to solve behavioural problems or to improve welfare. The enrichment programme should be designed proactively to prevent problems from arising, rather than only reacting to problems. It is also important to be aware that some activities that may seem to be enriching, when evaluated, may turn out to not be so.

Behavioural enrichment strategies should stem from our knowledge of the biology of the species in the wild (Shepherdson, Mellen & Hutchins, 1999). When considering the design of an environmental enrichment project, several points are worth considering that relate to elephant behaviour:

- **Sturdiness and safety:** Elephants, as physically strong animals, require enrichment which is resistant to damage and safe for them to interact with. This may change with life stage (e.g. modifications may be needed to make enrichment safe for calf interaction).
- **Control/choice:** Animals may benefit greatly if they have some form of control and choice over their environment. Control and choice have been identified as a crucial element of positive welfare across a wide range of species (e.g. Kurtycz, 2015).
- **Reinforcement:** Enrichment devices will only work over the long term if the attraction of the device lasts beyond the initial period of novelty. Reinforcement is critical and the elephant needs to receive a positive experience from the interaction. This is most obvious with food rewards; for example, an animal may play with a ball for the first five minutes or so and soon ignore it, however a ball that delivers a feed reward during play will hold the animal's attention for far longer. Other (non-food) rewards should also be utilised where possible. Effectiveness of rewards is specific to individuals and may change with life stage or social status.
- **Predictability:** Positive stimuli occurring in an unpredictable fashion both temporally and spatially are of greater benefit than when it is predictable (e.g. Bassett & Buchanan-Smith, 2007).
- **Neophobia:** On the other hand, animals might exhibit fear (e.g. demonstrated through alarm behaviours, avoidance behaviours) of new objects, particularly those that are completely unfamiliar to them. This can mean that novel enrichment items are at first ignored, or may even illicit stress or fear responses. These responses will vary according to individual personality and herd social dynamics. Initial neophobia should not prevent the implementation of a broad and diverse enrichment programme, however it is important that responses to enrichment are monitored and evaluated. Often neophobia will decline over time as animals are exposed to the novel item and given the opportunity to explore it in their own time. Such experiences may also be important for building individual and herd resilience to minor stressors as they provide opportunities to experience and learn to cope with novel situations and stimuli.
- Any enrichment should be set up in such a way as to **avoid competition within the herd** (e.g. it must be accessible by multiple individuals).

Goals for an enrichment programme can vary from broad aims, such as occupying time, to trying to increase a specific behaviour (see Table 9). Whatever the selected goal, it should have a direct benefit to the animal and encourage species-appropriate behaviours or reduce undesirable behaviours. In addition, an enrichment programme goal may also focus on other elements of zoo husbandry such as increasing public visibility and visitor dwell time for a species, as long as it also promotes good animal welfare.

Behavioural goal	**Enrichment provided**	**Equipment needed**
Increase positive social behaviours between individuals	Activities which allow elephants to interact together e.g. large wallows, sand mounds, pools, large browse piles etc.	Normal elephant resources arranged in such a way to encourage elephants to socially interact in a positive way, e.g. large browse piles to encourage group feeding, sand mounds arranged to encourage appropriate group sleeping etc.
To encourage elephants to use their trunks more, and increase feeding time through cognitive puzzle solving	Multiple feeding devices that encourage trunk manipulation e.g. hanging long tubes filled with variety of food with holes for browse to be inserted requiring problem solving and experimenting to be able to remove it. Hanging fire hose weaver with fruit pushed into the gaps.	Puzzle feeders, browse, hay, fire hose
To encourage cognitive thought	Appropriate positive reinforcement training of behaviours required for health and welfare monitoring.	Training plan for each elephant that contains discreet individual training goals specific to the needs of that animal.
Encourage species specific behaviours such as sand bathing or dusting to promote good skin health	Sand to dust with	Sand and water (hose/sprinkler)
Encourage species specific behaviours such as bark stripping	Fresh cut large trees stood in indoor or outdoor enclosure.	Fresh cut large trees
Encourage species specific behaviour such as mud wallowing to provide exfoliation and protection to elephant skin	Mud wallow that is accessible to elephants with mud that is appropriate for purpose and not too sticky.	Mud wallow, water

Encourage physical exercise to build trunk musculature	Suspended hay nets at varying heights.	Hay nets on automatic and manual winches
To encourage species specific behaviours such as bathing and swimming	Pool that is deep and large enough with appropriate entry and exit to encourage all animals to use.	Pool
Encourage exercise and physical activity	Variety of substrates which can be routinely moved around to alter topography.	Substrates, machinery to sculpt topography
Encourage species specific behaviours such as scratching to help to exfoliate skin	Large brush heads attached to objects in enclosure. Large rocks which can be routinely moved.	Large brush heads, large rocks, heat lamps and elephant-controlled showers
To encourage elephants to use novel objects and express natural behaviours such as scratching	Hanging objects	Trees, fire hose, rubber balls, barrels, broom heads, vertical mobile tree trunks
To encourage smelling behaviour and investigation of faeces with trunks	Provision of faeces, especially beneficial prior to introduction of a new elephant or breeding	Faeces from another elephant
Increase foraging time and encourage the elephants to walk and use more of the enclosure	Natural substrates such as grass, can be manipulated by special grass and herb mix. Scatter feed	Food taken from daily diet
Increase foraging time, encourage elephants to display natural feeding behaviours	Large tree trunks added to their enclosure frequently	Large tree trunks
Increase foraging time, reduce anticipatory behaviour	Big browse pile that allows for social feeding and multiple re-visit opportunities	Large quantity of browse
Increase feeding times and encourage digging behaviour	Bury food in the sand, either directly or within tubes.	Hand shovel, paper bags, cardboard tubes and digger

Increasing feeding time for the individual elephants to reduce anticipatory behaviour around electric fence. Increase trunk manipulation.	Large boomer balls in low or high feeders/ hanging plastic barrows filled with higher value food items.	Large boomer balls, training biscuits
To encourage elephants to display natural feeding behaviours, increase feeding time and reduce anticipatory behaviour in a specific part of enclosure	Hanging tyre with fire hose weaved across tyre. Preferably do not use a chain, as several bulls have fractured a tusk while playing with a tire on a chain. Fill with hay and a variety of other food they enjoy.	Fire hose, hay, training biscuits

Table 9 Examples of goal driven enrichment items for elephants

2.6. HANDLING

2.6.1. INDIVIDUAL IDENTIFICATION AND SEXING

In addition to the use of microchips (implantation subcutaneously behind the left ear; Fig. 16), elephants provide a variety of visual characteristics allowing an individual´s identification. Examples for the latter are as follows:

- shape of the pinnae in combination with unique slits or tears that are often present and/or characteristic conformation of the margins (Fig. 17).
- shape and conformation of the tusks (Fig. 18).
- shape of the backbone (Fig. 19).
- conformation of the tail end (Fig. 20).
- depigmentation patterns (exclusively in Asian elephants, Fig. 21).

According to our experience, most individuals can be easily identified based on these traits.

Fig. 16 Location for the implantation of a microchip behind the left ear.

Fig. 17 Shape of the pinnae with individually unique slits or tears.

Fig. 18 Shape and conformation or absence of tusks often represents a unique individual characteristic.

Fig. 19 The shape of the backbone is highly variable, particularly in Asian elephants.

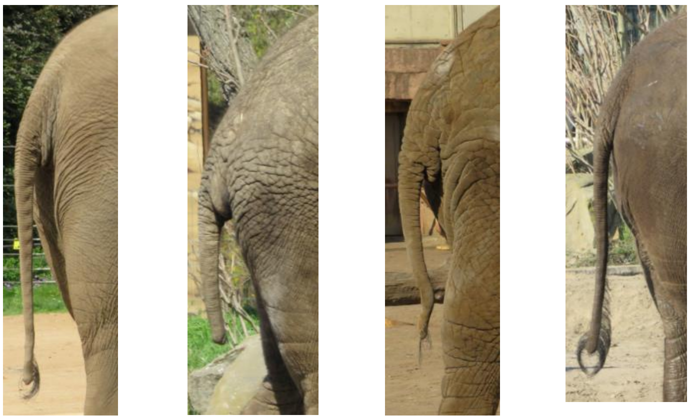


Fig. 20 Various conformations of the tail end.

Fig. 21 Depigmentation patterns are mainly present in the head region and occur exclusively in Asian elephants.

The African as well as Asian elephant species express a pronounced sexual dimorphism (see also the morphological data given in section **1.2 Morphology**). Being aware of the corresponding characteristics is very helpful if the urogenital opening cannot be inspected from up close or when evaluating a pictorial document. The sex-specific characteristics may become pronounced only in adult elephants and are not yet obvious in juvenile and sub-adult individuals.

Visually detectable sex-specific characteristics include:

- conformation of the urogenital opening (Fig. 22)

- shape of the forehead (Fig. 23)

- shape of the trunk basis and tusk conformation (Fig. 24)

Exceptions of these patterns may occur.

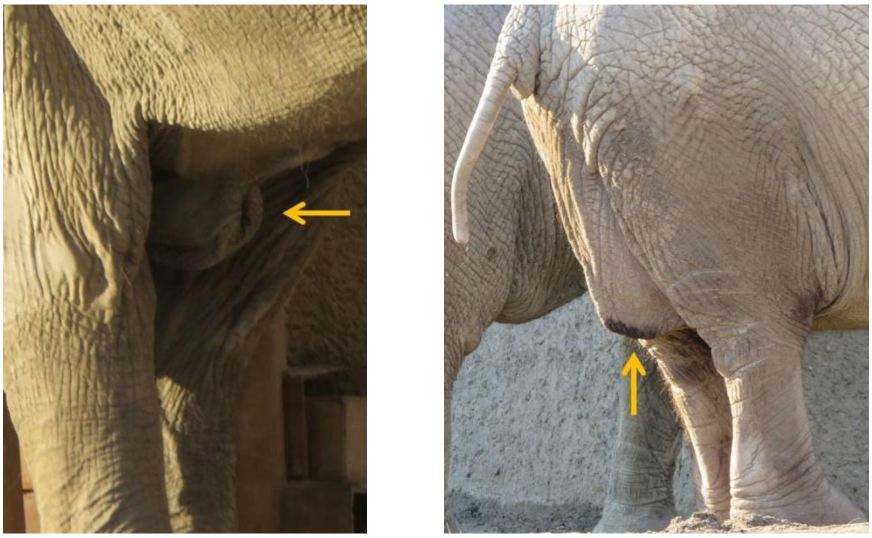


Fig. 22 In male elephants the urogenital opening has a round shape and is oriented cranially (parallel to the ventral abdominal wall; see left). In females the opening is oriented more distally and has a pointed, oval shape (see right).

Fig. 23 In African elephants the shape of the forehead is less rounded in females (a) compared to males (b). Asian female elephants do show much less prominent domes on their forehead (c) compared to males (d). In general, the skull shape is more pronounced in males compared to females.

Fig. 24 In male elephants (b, d), the trunk base, including the sulci of the tusks is of similar width to the interocular distance and tusks grow in a laterally orientated direction. Contrastingly the shape of the trunk base is far less pronounced in female elephants and tusks grow nearly parallel to the trunk (a, c).

Photo credit in this chapter: where not indicated differently copyright Christian Schiffmann.

2.6.2 General handling

The only recommended method of handling elephants in zoos is via **Protected Contact (PC).**

What is Protected Contact?

Protected contact is a style of management where all contact with the elephant is performed through a purposefully built protective barrier. Protected contact training is achieved through positive reinforcement techniques using targets, food rewards, body positioning and the voluntary participation by the elephant. The elephant and elephant staff do not share the same space; except under certain conditions where restricted contact (RC) is required as a management tool within a protected contact system.

Direct contact with an elephant in restraints (ropes or chains) is not considered protected contact; it is restricted contact. This is when an elephant, for example, is put in four restraints during a specific procedure such as, but not limited to, EEHV treatment of calves, transport or rectal examination in an elephant restraint device (ERD) or artificial insemination (AI).

Restricted Contact procedures must be individuallyassessed for risk and carried out by experienced elephant staff.

Why is Protected contact Modern Elephant Management?

Protected contact is beneficial for the safety of both elephants and responsible staff as it removes any necessity for the keepers to maintain a socially dominant relationship over the elephants (Desmond & Laule, 1993). This may have social implications, for example, by encouraging positive social cohesion, increased relationship bonds between the elephants and an increased behavioural repertoire, as negative consequences to behavioural expression are removed (Desmond & Laule, 1993, Veasey 2006). Additionally, reduction in aggression and conflict between elephants have been reported (Desmond & Laule, 1993; Maddox, 1992).

For more details see *"EAZA position statement on the Evolution of Elephant Management Systems at Member Zoos."* at **EAZA Elephant TAG SharePoint**.

Principles of protected contact

- The elephant can exhibit choice over participation with training.
- Positive reinforcement is the primary technique used.
- In case of undesirable behaviour, ignoring the behaviour is the most effective method of response. Implementing a "time out" may reduce uncooperative behaviour of the elephant. This is the only form of "punishment" that is acceptable within a PC system. Physical punishments for non-compliance are unacceptable.

PC tools

- **Body language**: elephants will take cues off of the trainer's position, posture and behaviour.
- **Training targets**: targets should be lightweight with an appropriate length to ensure it is possible to maintain distance between keepers and the elephants they are working with. Generally, targets are between 1,2m – 1,6m long although longer or shorter variations are appropriate depending on the access an elephant has to keepers through any protected contact barrier.
- **Positive reinforcement**: positive reinforcement via rewards is the primary technique (e.g. food) and should be correctly presented to encourage development of desired behaviours.
- **Bridge**: a verbal cue, clicker or, in some cases, whistle are all examples of acceptable bridges.

- **The PC wall**: beneficial to achieving healthcare standards in PC. Consider the following when designing the wall:
    - Positioning
    - Port position and flexibility (animal comfort)
    - Structure of the elephant group – e.g. calves with mothers, height of foot ports for older animals
    - Visibility between elephant and trainer
- **Protected contact walls:** The wall must be of appropriate dimensions to allow the elephant to be moved and positioned in order to suit the behaviour being trained. Dimensions of 9m long and 3,5m high (up to 4m for an adult bull) are recommended.
- The inclusion of a "dogleg" in the wall allows for easier access to back feet.
- Multiple walls and wall lengths will give more flexibility during the creation of husbandry routines.
- Trunk versatility should be considered: African elephants are more versatile with their trunks and can pass it through relatively tight areas. Asian elephants are less flexible with their trunks and so access space for conducting behaviours can be slightly wider.
- Areas to which the elephant can reach should be clearly marked and all persons that enter those areas in the presence of an elephant should be either appropriately trained staff or under supervision of appropriately trained staff.

Figure 25 Diagonal bars radiating from centre of the PC wall ©Pavel Vlček

For more advice please contact the TAG and/or training advisors of the TAG.

Staff safety around PC walls

Designated safety lines should be in place at all PC barriers. The safety protocols developed should ensure that working within these lines requires maximum concentration and focus on the elephants' demeanour and position. These areas must be tidy, and surfaces must not present a slip risk even when wet.

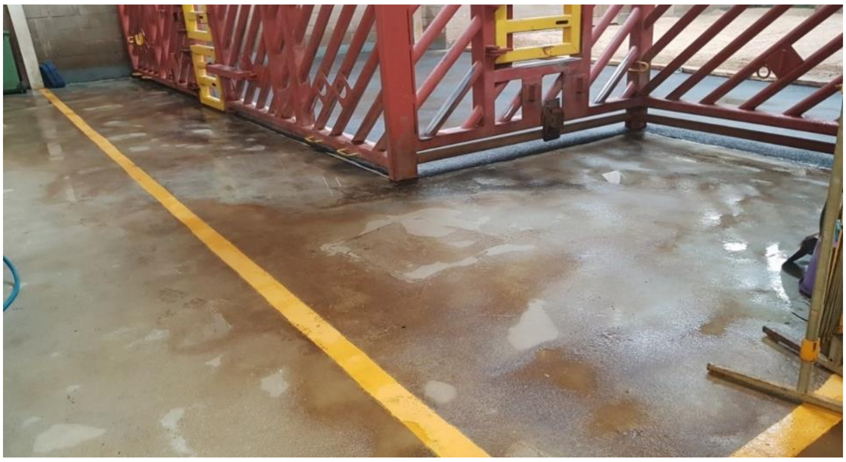


Figure 26 Clear lines of demarcation around the PC wall

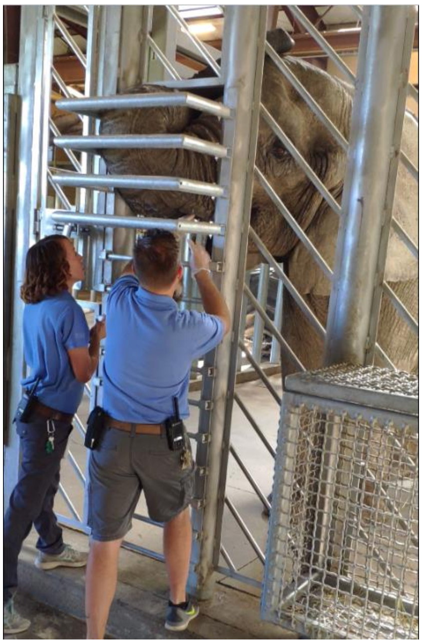


Figure 27 Specially designed mouth and tusk inspection windows, to maintain fuctional and safe health check procedures in high risk situations ©Martin Kristen.

Theory of training

Behavioural science shows that the two types of associative learning applied most successfully to animal training are operant conditioning and classical (Pavlovian) conditioning. All animals learn through both methods. Operant conditioning is used to teach a new behaviour, however, trainers should always be aware that classical conditioning would be occurring concurrently.

Definitions

Classical conditioning: the animal learns the relationship between two stimuli as predicting something pleasant or aversive. The animal is not learning a new behaviour but will respond in a reflexive way to the stimuli. A neutral stimulus, initially incapable of eliciting reflexive responses (behaviours), acquires the ability to do so through repeated pairing with other stimuli that are able to elicit such responses. This type of conditioning does not involve any voluntary choices by the animal and is described as a "stimulus-response" relationship, as the behaviour is automatically triggered by the stimulus.

Operant conditioning: the animal learns the association between its actions (behaviour) and the consequences. There are two main types of consequences, reinforcing (strengthening) and punishing (weakening). A behaviour is strengthened if followed by the addition of a stimulus the animal desires (known as positive reinforcement) or the removal of a stimulus the animal does not desire (negative reinforcement). Likewise, behaviour is weakened if followed by the addition of a stimulus the animal does not desire (positive punishment) or the removal of a stimulus the animal desires (negative punishment). The animal learns which behaviours enable it to best succeed at gaining positive reinforcers, either by acquiring pleasant, desired stimuli or avoiding unpleasant stimuli. Thus, the animal "operates" on the environment, leading to a desired outcome. This type of learning is described as a "response-stimulus" relationship, as the behaviour is not automatically triggered by the stimulus.

The most common term in zoos that referes to the process of teaching new behaviours through operant conditioning is "Positive Reinforcement Training" (PRT). PRT is an important management tool, and has many benefits for use in zoos (Desmond and Laule, 1993; Innes and McBride, 2008; Pryor, 2002; Laule and Desmond, 1998; Hare and Sevenich, 2001). It is important to remember that every interaction between an animal and a human is a training experience. This is particularly relevant for animal caregivers who should be consistent in the use of PRT methods in all of their interactions, whether they are formal training scenarios or not. Other forms of learning are also utilised in animal training programmes, notably habituation and desensitisation, with or without counter-conditioning (Pearce, 2008).

Reasons for training

- Improved husbandry
- Enrichment for the elephant as training can be used to increase activity levels and solve specific behavioural issues (Ramirez, 1999).
- Improved veterinary access

- Safe working practices
- Live animal demonstrations and events (see Demonstration guidelines in Appendix IV)
- Supporting development of conservation technology and research projects
- Achieve conservation education goals

Training programme in general

The training programme should give information on:

- Overarching aim/goals of the programme (e.g. veterinary access, conservation education)
- Identify trainers (staff)
- Identify individual elephant (see Individual training plan below)
- Identify how training is evaluated and reviewed
- Risk assessments for training programme

Individual training plan

The individual training plan should be designed specifically for each animal. As a living document, the individual training plan requires regular review (at least annually) and should consist of itemised, specific training goals. The training plan should specify behaviours that each individual requires which may vary between individuals, depending upon situations such as health status. The individual plan should cover how health monitoring will be achieved for each animal specifically, through a series of training goals. Training plans should account for life stage of the elephant.

Behaviours which adult elephants (over 8 years) should be trained for:

- Examination of integument, mouth, ears, eyes, feet
- Ability to perform foot care as required
- Body weight and body condition scoring, in line with protocols
- Health monitoring photographs (e.g. body and feet) and scoring
- Intravenous access
- Rectal access (including for ultrasonography)
- Acceptance of intramuscular injections
- Acceptance of ultrasound examination
- Trunk washes for disease screening and clinical monitoring (e.g. tuberculosis, herpes virus)
- Restraining
- Crate training

Calf training

Daily positive interactions, even if only a few moments, can begin very early in the animal's life given the correct infrastructure and can provide a good foundation for later training. From birth, desensitisation to presence of keepers, including via smelling, touching and playing next to keepers, builds trust.

Structured training should start at about 4 months of age. Training must fit into a regular slot during the morning routine and it should not exceed more than 30 minutes per session. Creating positive relationships between staff and young elephants can be very quickly compromised and the desired outcomes should never go beyond the young animal's capability or attention span, thus an approach of short but frequent sessions is often best.

Confidence of the mother and that of the calf in the training process must be maintained. In initial stages, the calf must always be able to return to its mother, and at later stages, at least be able to keep contact with her. Attempting to close doors or bars will be an issue if the young elephant is not ready to progress. The areas for contact with calves are crucial, and flexible barriers within the adult areas have proven very effective to promote the increased contact required.

Behaviours which calf (1-8 years) should be trained for:

- Examination of integument, mouth, ears, eyes, feet
- Body weight and body condition scoring, in line with monitoring protocols
- Health monitoring photographs (e.g. body and feet) and scoring
- Intravenous access
- Rectal access
- Acceptance of intramuscular injections
- Acceptance of ultrasound examination
- Trunk washes for disease screening and clinical monitoring (e.g. tuberculosis, herpes virus)

Blood sampling

The use of 10% lidocaine creams has improved the comfort of the elephant during blood sampling procedures, and the use of small gauge 0.3ml diabetes needles has worked well for regular EEHV detection sampling. Using microwaved beanbags to warm the back of ears for a few minutes increases the chances of a good sample using needles or lancets but does appear to increase the sensitivity for the elephant.

Rectal access

Allowing the tail to be handled and initial access to the rectum can be started from an early age. It should be possible to lubricate and insert a small diameter soft tube (25 – 45cm) into the rectum for administration of antiviral drugs in solution if required by 10-12 months. The dose rate may require slight increases to contend with some absorption into the faecal matter and solution volumes should be low (100ml or less) to reduce the chance of the drugs being pushed out. Administration after the animal has defecated is also an advantage. The traditional raking out of faecal matter is unlikely to be safely possible within the timescales required for herpes remediation. The use of restraints to hold elephants in position will be unlikely to yield positive results, and training may be an ineffective use of the time spent with the calf.

Sub cutaneous injection

It is possible to inject small volumes (2-4ml) sub-cutaneously with the correct preparation and experience and in the appropriate circumstances. Large needles should be avoided in young animals and positioning away from thick skin areas will assist in delivery.

Oral inspection

Is possible from an early age (two to four months) to conduct an oral inspection and it becomes easier as the calf begins to become interested in food items, usually between three to five months. This allows for daily temperature collections using a laser thermometer to build up a pattern of normal range for the calf. Desensitisation to torches and camera flashes is also beneficial.

Weigh bridge

It is possible to use the adult weighing scales to weigh the calf. For Asian elephant calves, especially when treating EEHV, the installation of an accurate weighbridge displaying 1kg increments should be included in the calf treatment area as the depression of growth may be an early indicator of an issue for the young animals.

EEHV treatment

Further information on EEHV treatment can be found in chapter **2.7 Veterinary**

Basic husbandry behaviours for working elephants in Protected Contact (PC)

The use of a positive reinforcement training technique is common to all elephant training in a PC setting.

The following recommendations can be useful when starting to train elephants in PC:

a. When starting to work in PC the trainer/keeper must understand the basic principles of positive reinforcement training.

b. A clicker, whistle or verbal bridge is used to confirm or reinforce the desired behaviour. It is important to know exactly when to apply the bridge- timing is key for successful training.

c. Before starting a training session, make sure all necessary equipment is ready: food (or other) rewards, targets and clicker/whistle.

d. Start with conditioning the bridge by pairing the clicker/whistle/voice with the reward during 10 minute sessions, multiple times a day. Then continue with an attention exercise (meaning walk from left to right to see if the elephant follows the trainer/keeper). If the animal shows attention and follows, then apply the bridge and reward the behaviour. Attention is another key for successful training.

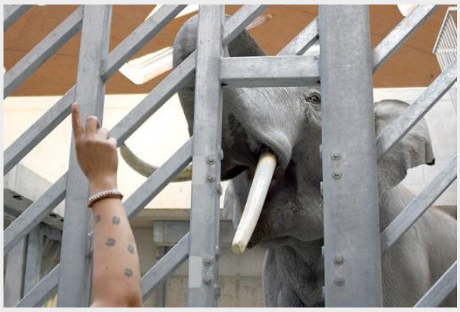
e. Once the bridge is established, the trainer/keeper can start training different behaviours.

The following list contains the most important cues and commands for carrying out husbandry and medical procedures in PC. Depending on institutional requirements, additional cues might be used (such as 'take', 'give', 'go target', 'knee', 'blow', 'head down' etc.)

Cue/ Command	Behaviour/ Comments
TARGET	Prerequisite for other behaviours Elephant stands with the forehead against the target. Exact target location may vary among institutions but is usually approximately 20 cm below or above the eyes.  
COME HERE	Elephant comes to trainer/keeper
OKAY/ RELEASE	Release, end of exercise After every cue/command that is performed correctly, the term "Okay" or "Release" is used to signal to the elephant when a desired behaviour is completed.
STEADY	The elephant holds (freezes) the current position.

GO ON	The elephant moves forward in straight line until "steady" follows.	
GO BACK	The elephant moves backwards in straight line until "steady" follows.	
STRAIGHT	The elephant positions itself with the head towards the trainer.	
SIDE/ MOVE IN/ LEAN IN	The elephant positions itself along the fence, presenting the full side to the trainer/keeper. With a second target the elephant is given the information which side to present, right or left.	
TURN/ GO ROUND	Usually this cue is used to gain access to the back side of the elephant. Starting from a "side"- or "straight"-position, the elephant turns into a position, facing 180° away from the trainer.	
FOOT	The elephant presents left or right back foot through the foot hole in the training wall.	
FOOT	The elephant presents left or right front foot through the foot hole in the training wall. It is best to work with both hands: Left hand with target on head, with right hand target the trainer asks for left front foot. Hands switched accordingly for the right foot. Targets are used for the correct positioning of the foot (small scale movements) within the hole.	

MOVE OVER	Head is on target and the back of elephant will move to left or right, according to the position of the keeper.	 
OPEN	The elephant opens its mouth wide enough to present the molars.	
TONGUE	Elephant presents its tongue	
TRUNK	Elephant presents the tip of trunk in the hand of trainer (trunk goes under the wrist into the hand).	

TRUNK UP	The elephant lifts its trunk over the head (e.g. for trunk washes)	
DOWN	The elephant lies down in a frontal and straight position.	
LIE DOWN	The elephant lays on its side, left or right, according to the position of the keeper.	
TAIL	The elephant presents its tail in the hand of the keeper. This can be from "side" position or from the "turn" position.	
EAR	The elephant presents both ears towards the training wall e.g. for inspection.	

EAR	The elephant presents one ear through the training wall e.g. for blood collection.	

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2.6.3 Restraining

This may be required for new or more invasive veterinary procedures, possibly under a standing sedation, or within an ERD. Restraint is also required during transport. It may be necessary to restrict the animal's movement to ensure the safety of all involved.

Elephants must be trained to accept restraints slowly; details of how this is achieved should be given in the animal's training plan.

- **Ownership**: All restraint equipment should be acquired and maintained to a specification agreed by zoo staff. Any wear and tear, which might cause weaknesses in equipment and therefore pose a safety risk, or possibly lead to injury to elephants, should be avoided.
- **Authorisation for use and prohibitions**: The current zoo director, responsible senior manager or curator should give generic approval of routine restraint.
- **Elephants must not be routinely restrained for periods in excess of one out of 24 hours** (unless in justified cases, e.g. loading).
- **Accountable individuals and records**: Only trained persons may carry out restraint. This may include elephant experts brought in for staff training and/or elephant transportation.
- **Training**: Keepers must be adequately trained in the procedure and safety aspects followed; training should be documented.

2.6.4 Transportation

All elephant transports must be conducted in line with the **EAZA Elephant TAG transport protocol** (available online at **EAZA Elephant TAG SharePoint**) with relevant pre-transport health screening and only **with TAG/EEP approval**.

Sending institutions are recommended to visit receiving institutions prior to confirming transport agreement. This enables good preparation for the transport and acclimatization of the transported individual. Welfare, suitability of enclosure design and safety, and staff ability/training can be assessed and addressed first-hand and assurances put in place before moving the animal. Keepers from the receiving collection must spend time working with the elephant at its home institution prior to the move in order to get to know the animal and its usual husbandry routines. Keepers from the donating institution must accompany the elephant during the trip and stay to assist with acclimatisation following the move.

Institutions should consider, in advance of the move, how they will communicate information to the media and the public.

Paperwork

CITES: required paperwork for moves. CITES officers liaise to confirm the receiving institution can accept the animal, and in turn they talk to both institutions, demonstrating due diligence on their part. CITES can stop the movement if they find the transfer inappropriate and will not issue the necessary permit.

TRACES ITAHC (Intra Trade Animal Health Certificate) or CVED (for 3rd country exports): required paperwork at time of writing. This is not just a health certificate to state the animal is fit to travel, it is also a means to alert borders of what, when, how and who is crossing their border. If required, the shipment can be stopped at the border and redirected. For example, if the country to be driven into had an outbreak of foot and mouth disease, an elephant would be stopped from entering and an alternative plan would have to be enacted.

Preliminary considerations

- Assessment of elephants, including age, sex, temperament and degree of training as well as veterinary history and any health issues should be discussed prior to transport.
- The places of loading and unloading (access to elephant house) must be assessed prior to the move. For loading and unloading of the crate, a crane will be required, as with the transporter, use of a reputable company is recommended. A recent weight of the animal and the crate is required to ensure a suitable crane is used. Crane companies should be informed that a moving animal in a crate brings more carrying weight than a motionless object.
- Suitability of elephant house/yard for loading and unloading elephants, i.e. elephant and keeper safety. Adjustments to the facilities might be necessary prior to the move. Current equipment must be assessed for suitability in the assistance of loading and unloading elephants, i.e. restraint equipment.
- Animal should be acclimatised to the transport crate and trained to enter it voluntarily. This may take some time, particularly with animals managed in a protected contact system, and consideration should be made to having the crates in situ for a period of time preceding the transport to allow for regular training. Although this can be costly, it is tremendously helpful. Elephants should also be acclimatised to the placement of leg restraints. In groups where regular elephant moves are expected, for example collections holding young bachelor males, crate and restraint training should form part of the regular training routine.

Loading and unloading elephants

- Winches for loading should be avoided. If necessary, winches should only be carried out by a very experienced operator with full and appropriate risk assessment.
- Where animals are loaded without the use of winches, only the usual keepers should be present until the elephant is restrained inside the crate. The process should be carried out in

the same way as a normal training session. Additional help should be close at hand and in radio contact.

- The positioning of other equipment such as cranes should be considered. Where these are visible to the elephants during loading, it may be beneficial for them to arrive 1-2 days earlier so that the animals have time to become acclimatised to their position. An alternative would be to position them after the animal is loaded in crate.
- Collections should have a clear and pre-discussed plan in place for what will happen if the animal refuses to load on the day of transport, whether the transport will be postponed or other actions such as sedation or winching carried out.
- It is recommended that a veterinary surgeon is present during loading, under loading and throughout the transport in case of emergency.

Use of restraint

Elephants in the transporter must be restricted at all times to ensure they travel securely and cannot move and cause damage to the crate. This is accomplished by attaching restraints to legs of the elephant. These must be fitted correctly, be at the correct length and be tight enough to restrict the animals but not uncomfortable or so tight that they interfere with the elephant's balance. The elephant must be able to move forwards and backwards. Factors to be considered are:

- Females are normally restrained in front and behind on opposite sides, i.e. on two feet. Males are best restrained on all four legs. One chain is not sufficient due to risk of transported animal to turn around in the crate or move excessively.
- Restraints must be secured on the outside of the crate to allow them to be released in an emergency, without entering the crate.

Staff safety

Even experienced elephant keepers may not have had much experience in transporting animals, therefore, a full briefing must be carried out prior to moving the animals. All risk assessments must be carried out and detailed for each staff member and each elephant.

Elephant welfare during transportation

- Suitable locations for regular stops should be planned, both for driver rest periods and for elephants to be rested and given food and water. Hay and browse should be supplied regularly to occupy the animals during the journey. Overnight stops should be planned at a zoo wherever possible, for supplies of fresh food and water and for security reasons.
- Suitable collections, along or close to the route, to which a diversion could be made in an emergency should be planned in advance. Up-to-date contact details of all emergency contacts should be carried for the duration of the journey.
- The size and height of the vehicle and crate should be recorded, and route checked to ensure accessibility.

- There should be provision for heating or cooling the animals during transport.
- Transport crates should be fitted with cameras capable of recording during low light (if driving during evening/night). The camera must feed to a member of staff travelling directly with the elephant so they can always be monitored.

2.6.5 SAFETY

Staff training

An effective programme for staff training is required. While much progress has been made within the elephant community regarding the individuality and specialist nature of elephants and therefore the need for them to be treated differently, there must be recognition of the cognitive ability and strength of these animals, as well as the species-specific needs.

An effective staff training programme must be built around risk assessments carried out on all operating procedures. The process of risk assessment informing documented operating procedures and systems of work, with regular revision and updates, leads to the development of a dynamic training programme allowing for progressive working practises.

A minimum of two appropriately trained elephant keepers must be present during any contact with elephants, even if they may not both need to be present at the PC wall/station at the same time. A collection should have a written and monitored staff training programme. This should encompass the training of new staff, with a logical progression of skills, as well as training reviews of existing staff. A training programme should be developed from an analysis of the current facilities, and the existing staff ability. The training programme should also measure implementation with a list of targets (task achievements) against which staff progress can be measured.

Elephant keepers must be properly and consistently trained for the duties and tasks they are expected to undertake. The elephant keepers work in a defined team with a structure which includes a team leader who has the responsibility for ensuring that systems of work are implemented, documented and reviewed.

The length of training for new keepers will be dependent on the facility and on the experience of the new and the existing teams.

Protocol

A complete list of jobs (tasks) and environmental factors should be compiled in a protocol for each elephant and include:

- Risk assessments
- Event of keeper being inadvertently inside with elephant (escape routes, emergency procedures for all)
- List of PC procedures with elephant
- Restraint (using straps, webbing or chains)

- In restraint for veterinary procedures
- Gates and gate controls
- Nutrition
- Equipment and materials which would be used
- Cover and environmental enrichment features
- Temporary features
- Zoonotic risks
- Safety protocol

2.7 VETERINARY: CONSIDERATIONS FOR HEALTH AND WELFARE

These recommendations are written for the benefit of elephant keepers and are not intended to be a comprehensive veterinary reference document.

Similarly, this chapter will not cover all disease conditions that can affect elephants, but the more relevant ones which keepers and vets may encounter in the care of their animals. Textbooks and references are available for more detailed information.

Preventive and proactive health care

Observations

Twice daily observations at each end of day of each elephant must occur for signs of anything physically or behaviourally abnormal and appropriate records kept using ZIMS (or other recognised record management software). Any concerns or abnormalities must be swiftly followed up as appropriate. Reporting systems must be in place to allow veterinary input immediately.

Weighing

Weight should be measured for each individual. Weight change can be the first indication of a health issue. It is recommended that calves are weighed weekly. Results of weighing should always be combined with body condition scoring (see Body condition scoring guidance in Appendix V or available online at **EAZA Elephant TAG SharePoint**) as the weight itself might be misleading and health condition should always be interpreted as combination of both weight and body scoring. Diet should be reviewed annually and adjusted when required in response to weight changes in individual elephants.

Foot care

Routine foot care must be carried out on a case by case basis, as required. Any foot problems must be managed as soon as possible, and preventive measures initiated to reduce the incidence. See **2.8.1 Foot Care** for more detailed information.

Faecal testing

Routine, individual faecal sampling is advisable. Samples should be collected over a three to five day period and analysed for any bacteria or parasites that could cause disease. The frequency of testing is dependent upon the history of such findings in the group but should occur at least twice a year. Treatment should be initiated dependent on results. Dried maize (not flaked) or edible glitter can be fed to identify an individual elephant’s faeces. Checking CCTV footage where available might also help.

Urine sampling

Urine samples can also be used for checking the presence of blood, glucose, protein, pH measurement, hormone analysis (for welfare or reproductive assessment) and many other health factors.

Blood sampling

For standard analysis, blood sampling is recommended twice yearly from the age of one year on to create a database of normal values for each individual. Subtle changes in the blood can be very informative when trying to diagnose disease (e.g. in calves with active EEHV infections). It is advisable that breeding herds are banking plasma and serum at -20°C for EEHV treatment; these blood products are thought to increase the survival rates of treated calves.

Identification

Microchips should be used with the standard position behind the left ear, under the skin.

Vaccination

Vaccine programmes vary based on the history of disease within the collection and the risks in that geographic area. The veterinary advisors of the elephant TAG have recommended to vaccinate Asian and African elephants against cowpox (see Advice MVA vaccinations elephants March 2018 in Appendix VI). Some collections also vaccinate yearly against tetanus and/or other clostridia.

Measurement of body temperature

Temperature measurement in the rectum or in fresh faecal bolus (within 5-9 mins of excretion) can be used, although comparison between individuals is advised. Faecal bolus temperatures are thought to be 0,5ºC higher than rectal temperatures. Some collections use infrared thermometers to record the temperature of the roof of the mouth, which will be lower than core body temperature, but may provide useful to inform trends in individuals.

Trunk wash

The ability to perform a trunk wash is a requirement. For this procedure, 60 ml of sterile saline is put into a nostril or nostrils, the trunk is raised and lowered to wash the saline up and down, and then the saline is forcibly expelled (by the elephant) into a sample bag placed over the end of the trunk.
When conducting trunk wash, the following needs to be considered:

- The trunk needs to go high enough to ensure saline reaches the upper portions of the trunk, therefore ideally raised above head height.
- The saline needs to be actively exhaled by the elephant, not allowed to come back out under gravity alone.

Elephant Down Protocol

Collections should have an elephant down protocol detailing response to an elephant that is down and in need of assistance. This should include a site-specific plan and contact details for all necessary external parties. This protocol should consider each individual animal and all the various locations in which this could occur.

Treatment and diagnostics

Medication

Generally, drugs are given at the same dose rates as for horses or ruminants like cattle, unless specific data are available for the elephant. Such data are limited but increasing. Readers should refer to the literature, e.g Fowler & Mikota (2008) and www.elephantcare.org.
Medication can be provided in different ways:

- **Oral:** Elephants have a very keen sense of taste and can detect drugs hidden in food items readily. Bran mixture routinely provided every day is the easiest way to hide drugs. Strongly flavoured items such as mint, molasses, fruits, and fruit juices can be used as carriers, and specifically flavoured drugs can be obtained. Elephants can be readily conditioned to accept a bite block for the direct administration of oral medications using syringes or by hand. When providing antibiotics orally, care should be taken to protect the digestive system microflora by adding probiotics into diet.
- **Rectal:** The rectal wall is very absorptive and has a large surface area so it can absorb some drugs very rapidly and effectively. It is a good way to give large volumes of fluids quickly. Administration via this route requires adequate training and/or chemical or physical restraint.
- **Intramuscular injection**: Muscles are well supplied with blood, so an injection into this tissue is also rapidly absorbed in most cases. Due to their body weight elephants require large volumes of drug and too large a volume at any one site can cause pain and predispose the animal to abscess formation.
- **Intravenous**: Injection directly into the blood means immediate distribution around the body at a known level. Inadvertent injection around a vessel can be very damaging to tissues however and should be avoided if possible.
- **Subcutaneous:** Injection under the skin is not recommended in elephants, as absorption is questionable and has not been studied. In general, it is not a recognised route due to the risk of abscess formation.
- **Topical therapy:** Topical treatments can consist of medication or the use of compresses (either cold or warm). These can be useful adjuncts to systemic therapy but maintaining them can be difficult with inquisitive elephants, which will readily remove them. Lavage is also useful in reducing contamination and bacterial load to any external wounds or abscesses. Lavage solutions include sterile saline, or diluted chlorhexidine or povidone iodine (see also information compiled in Table 10). Ophthalmic preparations can also be administered topically, with the choice of creams or drops tailored to the individual and the pharmaceutical agent required. Topical treatments such as silver sulfadiazine cream can be useful for wound management. Some institutions are now using low level laser therapy (LLLT) to assist with pain management, wound healing and infection prevention in a variety of lesions.

Radiography
Elephant calves can be radiographed as horses, but for adults, it is only practical at present to radiograph the extremities. This includes the ends of limbs, the tail, the trunk, and some parts of the mouth. Basic radiation safety and protection rules should always be followed. It is recommended that yearly radiographs are taken of adult elephant feet where any clinical conditions arise, to enable long-term monitoring. Baseline images can be extremely useful especially when assessing changes in the phalangeal bones (Mumby *et al*., 2013).

Thermal Imaging
Thermal imagine is a useful tool but can be open to over-interpretation, especially if not used at consistent times of day. A good review has been published by Hilsberg-Merz (2007). Necessary equipment for thermal imaging is usually owned by the local fire department.

Ultrasonography
Ultrasound is an extremely useful imaging tool for any elephant collection. It is used for reproductive assessment, pregnancy and parturition monitoring, but can also be used for general diagnostic procedures, as with other species, including lameness or eye conditions.

Sedation and Anaesthesia

This section is not intended to be a comprehensive guide and for more detailed information reference should be made to the veterinary texts listed in the reference list e.g. Fowler & Mikota (2008).

Planning is essential when performing any sedation or general anaesthesia. Appropriate numbers of trained and experienced staff present is essential, and a risk assessment should be performed. The area should be prepared in advance and additional equipment may need to be brought in. As for all procedures, anaesthesia is best performed in a quiet environment for smooth induction and recovery. There must be a plan for emergencies with appropriate drugs immediately available. Intravenous cannulation (i.e. immediate access to administer intravenous drugs) should be carried out as soon as safely possible to provide support during anaesthesia. Analgesia should be available as necessary.

Relevant Clinical Conditions

Tuberculosis (TB)
Screening for tuberculosis is recommended in line with the EAZA Elephant TAG TB testing policy (routine testing for all animals over four years old and pre-transport testing). See TB testing in EAZA captive elephants in the Elephant TAG in Appendix VII.

The gold standard test for TB in elephants remains culture and polymerase chain reaction (PCR) as these tests detect and grow any bacteria present. Culture and PCR are recommended to be performed on an annual basis on trunk wash (three samples taken within one week) or bronchial lavage samples on animals over the age of eight years old. It can take up to twelve weeks of attempted culture before a result is considered negative. Any suspicious lesions found in tissues at post-mortem examination should also undergo culture and PCR.

A negative result from culture and PCR on trunk wash/lavage samples means that an elephant has not been found to be shedding TB within the respiratory system at the time of sampling. A positive result from culture and PCR means that an elephant has active TB. Animals can have false negatives but not false positives from culture and PCR results.

Other tests for TB measure the response of the immune system, rather than the presence of the bacteria. As a result, these are hard to interpret, and not considered gold standard, but can still give useful additional information. Like all serological tests, for the diagnosis of tuberculosis in elephants, the gamma interferon test is an experimental test currently being performed by Utrecht University on elephant blood samples. All collections are requested to support this project by contributing samples. Validation of any immunological test will help us in future to identify exposed and infected animals, prior to the onset of shedding and will increase the sensitivity of our TB screening so we can make decisions early in the disease process.

It is recommended that a risk assessment be carried out for keeping staff and occupational health advice sought concerning routine screening of the keepers at respective collections.

Elephant Endotheliotropic Herpes Virus (EEHV)

EEHV is currently the most important, known viral infection in Asian elephants worldwide. It was identified by Richman *et al.* (1999) with studies then identifying cases as far back as the 1970s. This is not a new infection, as viral taxonomy indicates that the virus dates back at least 30 million years (Zong *et al.*, 2007). EEHV has been identified in range countries in both wild and captive African and Asian elephants (McCully *et al*., 1971; Reid *et al*., 2006; Zachariah *et al*., 2008) and is no longer thought to be due to close contact between the two species. There are currently twelve known variants of the EEHV virus (Long *et al.*, 2016).

Most, if not all, elephants carry EEHV, but only some develop clinical symptoms and become unwell; an understanding of why is imperative for future captive breeding and elephant conservation. Most cases in Asian elephant calves occur between the ages of one to three years old, although the risk period extends up to eight years old. Every collection with calves should have an EEHV protocol, which is reviewed at least annually, in line with continuous developments in disease detection and treatment. Screenings can be carried out on blood and a variety of other samples (e.g. trunk washes). The amount of virus in the blood stream will begin to rise at least two weeks before the onset of clinical signs (Long *et al.*, 2016) and before the virus is shed from the respiratory tract or elsewhere. The initiation of treatment prior to clinical signs is very likely to improve prognosis. It is recommended that blood is taken from calves at least weekly to check for the presence and quantity of virus, and look for characteristic changes to blood cells indicative of risk of disease.

Samples for diagnostic qPCR on EEHV can be sent to the following labs:
European labs that can perform EEHV qPCR
Date: 06-08-2020

Universität Berlin, Institut für Virologie
Robert von Ostertag-Str. 7-13, 14163 Berlin, Germany
Prof. Dr. Klaus Osterrieder/ Dr. Jakob Trimpert/ Dr. Azza Abdelgawad
Tel: (+49) 30 838 59780 / +49 30 838 67281
Email: **no.34@fu-berlin.de** / **trimpert.jakob@fu-berlin.de** / **azza.abdelgawad@fu-berlin.de**

Rotterdam Zoo, Veterinary Department.
Postbus 532, 3000AM Rotterdam, the Netherlands
Linda Bruins-van Sonsbeek DVM; Christine Kruger-Velema
Tel: (+31) 10 4431485 (+31) 10 4431541
Email: **l.van.sonsbeek@diergaardeblijdorp.nl** / **c.kruger-velema@diergaardeblijdorp.nl**

SCG Diagnosztika Kft / UVM Large Animal Diagnostic Centre
H-2225 Üllő, Dóra major, Hungary
Biksi Imre DVM ; Albert Ervin PhD; Dán Ádám PhD
Tel: (+36) 309487747
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EEHV specifically damages blood vessels, which results in internal haemorrhage. Clinical signs are all related to this including patches and blueness of the tongue, swelling of the head and forelimbs, ulceration of the hard palate, lack of coordination, lethargy, and mild colic. EEHV cases can be simply noted as a change in behaviour, and if this occurs in any animal under the age of 10 years then EEHV should be the primary diagnosis until proven otherwise. Clinical signs vary and can occur with the stress of concurrent disease. See Maluy & Hawke, 2007 and Garner *et al*., 2009.

Treatment is based on early and aggressive therapies including anti-herpes drugs, pain relief, antibiotics, fluids, and plasma transfusions. A recent management document has been produced by the TAG, which should be followed in all herds (**Recommendations for monitoring EEHV**). Another useful resource is eehvinfo.org, which details emerging best practice for treatment of the disease.

Musculoskeletal conditions

Knowledge of basic anatomy of the musculoskeletal system is essential to understand the normal function, disease processes, and supportive therapies that must be implemented to treat some of the most common problems seen in captive elephants. Good anatomical reviews are found in Lamps *et al.* (2001); Benz (2005); Weissengruber *et al*. (2006) and aspects of elephant locomotion can be found in Hutchinson *et al.* (2003), Hutchinson (2006), Miller *et al*. (2008), Ren & Hutchinson (2008). One obvious difference in elephants is that the fore feet are considerably different from the hind. Arthritis, gait changes, nail, and other foot problems in older animals need to be managed appropriately prior to welfare being compromised, and euthanasia is indicated as necessary.

Osteoarthritis

Osteoarthritis is one of the most common musculoskeletal diseases seen in captive elephants. The causes are multifactorial, as they are in other species, and include infection, conformational or gait abnormalities, nutrition-related disease early on in life, obesity, stereotypical behaviour, chronic mechanical insult (e.g. unsuitable enclosure flooring), and trauma (West, 2006). Factors that can potentially exacerbate or compound arthritis include lack of exercise, obesity, hard substrate, cold environmental temperatures and poor conformation. Lameness is the most apparent change seen due to arthritis, but regular monitoring of locomotion may provide earlier clues to problems. Often by the time arthritis is diagnosed, it can only be managed with analgesics, weight control, nutritional support, and exercise, including warm water hydrotherapy. If severe, then euthanasia may be advised. Routine locomotion and foot scoring should be used to assess affected animals and frequency of scoring may be increased as disease progresses. The use of CCTV for night behaviour study is required to monitor sleep patterns and the ability of individuals to lie down and rise from rest.

Trauma

Tail

Bite injuries can be seen in herds where aggression occurs between elephants. Open wounds can be managed by flushing with dilute iodine and followed by topical use of silver sulfadiazine cream. Radiography is recommended to rule out fractures and pain relief should be provided orally for deep lacerations or when the elephant is continuing to traumatise the area.

Trunk injuries

The trunk has a very extensive blood supply and thus severe haemorrhage may result following trauma. Paralysis of the trunk can arise from nerve damage. With the correct treatment, including feeding and management techniques, cases have been known to resolve over time.

Trunk paralysis

Partial or complete paralysis of the trunk with consequent muscle atrophy is occasionally seen in Asian elephants. The cause is unknown, but cranial nerve trauma or infection and arteriosclerosis have all been suggested. Flaccid trunk paralysis is a syndrome in its own right and is seen in African elephants in the wild, the cause of which is also unknown.

Colic

Colic refers to any abdominal pain and as such, can result from pain within an abdominal organ but, more often than not, it is in the gastrointestinal tract. Spasmodic colic, bloat, impaction, constipation, mesenteric tears, and infectious causes (including EEHV) have all been reported. Colic can occur after sudden dietary changes, and although not common, ingestion of large amounts of earth, sand, or stones can also cause problems. Colic can also be secondary to dental problems. Treatment is dependent upon the cause but symptomatic therapy including pain relief and fluid therapy are commonly used. Behaviours symptomatic of colic and labour can look similar. Behaviours indiciative of colic may include trunk kept in mouth, stretching hind legs, looking for support of a hind leg, crossing a hind leg while gnashing with the head kept lower than normal (Fig. 28).

Figure 28 Behavioural characteristics in two Asian elephants. A) Asian elephant in labour throwing sand at abdomen, keeping mouth wide open, lying down, and in a sitting position (© Willem Schaftenaar, Dak Lak Elephant Conservation Center, Vietnam). B) colic in Asian elephant. Trunk kept in mouth, stretching hind legs, looking for support of a hind leg, crossing a hind leg while gnashing with the head kept lower than normal (© Christian Schiffmann, Zoo Zürich).

Dental diseases
Conditions vary considerably and considerations to be taken include:

- Oral examination as an essential part of the daily routine for any husbandry programme.
- Deformities of the tusks; usually a result of trauma in early life.
- Excessive or abnormal wear of tusks rubbing on bars or cables can lead to sharp ends, weaknesses in the tusk shaft, and minor or major fractures. Sharp-ended tusks can cause severe injuries in conspecifics.
- Fractures and longitudinal splits of the tusk can compromise the pulp and bacteria can cause infection within the tusk or even into the maxilla. These conditions are not only life threatening to the elephant but can also be very painful. Simple and recent fractures involving the pulp may be reparable by immediate action and endodontic treatment, but more severe or chronic cases require extraction. Extraction is a specialist procedure, although basic tusk removal has been described (Steiner *et al*., 2003). Expert opinion must be obtained.
- Impacted molars often result from failure of the preceding molar fragments to be shed, which may be due to inadequate provision of browse. In extreme cases, 90-degree deviation of molars has been observed. Such molar problems can be a cause of chronic weight loss. Molar abscesses do occur and may progress to osteomyelitis and gross swelling of the mandible. Extraction has been described, but again requires specialist equipment and knowledge (Kertesz, 1993).
- Sulcus infections occur and are usually secondary to foreign bodies.

Ventral oedema (swelling under the skin of the underside of the elephant) has often been associated with a lack of protein (proteinemia) in the blood, electrolyte imbalances (potassium, calcium, phosphorus), or inflammatory processes (i.e. infectious diseases such as EEHV). It is also a common condition in late pregnancy and may remain until after parturition until resolution. A blood sample of the elephant should be analysed for complete blood cell count and serum chemistry, looking at inflammatory parameter (CRP, leucocytes), haematocrit, Fe, Ca, Mg, Na, P, as well as liver and kidney values. A full work up should be undertaken to determine the underlying cause. If the above named conditions are not the cause, a cardiac problem should also be excluded.

Chronic kidney failure has been found in several Asian elephants in recent years. Any renal disease is difficult to diagnose in elephants since normal blood kidney markers are not providing useful information about the elephant kidney (Lueders *et al*., 2019). Presenting symptoms may include weight loss, intermittent edema (abdominal, maxillary) and muscle fasciculations/convulsions (presenting as shiver or hick-up). At later stages, pale mucous membranes, generalized weakness and polydipsia develop. In four terminal cases histopathology confirmed chronic interstitial nephritis accompanied by lesions such as tubulo- or glomerulonephritis and mineralization; cystic degeneration; or pyelitis.

In early stages, haematology and biochemistry alone do not qualify as sensitive diagnostics. However, hypercalcemia, dysproteinemia and anemia (low HCT) consist common findings. Blood urea nitrogen (BUN) is usually the first of the renal enzymes to rise during course of disease, while serum Creatinine invariably remains unchanged until end-stage disease. In two elephants, after initial rise, BUN and SCrea even dropped dramatically prior to euthanasia.

Lowered urine creatinine (Urea) consistently is the most reliable clinicopathologic indicator of underlying renal disease. In a study on Asian elephants, UCrea remained significantly lower (mean ± SD: 0.09 ± 0.04 mg/ml; n=7) compared to unremarkable controls from the same facilities (0.26 ± 0.08 mg/ml; n= 5) (Lueders *et al*., 2019). All affected elephants displayed ultrasonographically visible kidney lesions. Transrectal ultrasonography revealed abnormal kidney outlines and size (n=3), renal cysts (n=5), prominent renal vasculature (n=5) and/or hyperechogenicity of the parenchyma with distal acoustic shadowing (n=5). In summary, a diagnotisc approach not limited to blood analysis but including measurements of UCrea and ultrasonography is beneficial for kidney function assessment. Treatment may consist of increased fluid supply (e.g. regular rectal enema, IV fluids), oral electrolyte supplements (calcium, potassium, phosphorus, iron), and low- protein food supplements.

Euthanasia and Post-Mortem Examination

The euthanasia of an elephant is a delicate procedure that is not only highly emotive but also involves potentially serious health and safety hazards. To ensure optimal safety of all members of staff involved, and to make sure that the welfare of the elephant is not compromised, the euthanasia event is broken into three components:

1. Planning and discussion to ensure that all parties understand that euthanasia is in the best interest of the animal and that all contingencies are made to ensure the smooth application of euthanasia.
2. Induction of general anaesthesia to ensure that the elephant is immobilised for the euthanasia procedure. This is in the interest of the animal's welfare primarily, but also provides safety for the team.
3. Once the elephant is recumbent then euthanasia should be administered. Assessment of death should be based on a combination of loss of electrocardiogram via a preplaced ECG, lack of cranial reflexes, lack of respiratory movements, and a lack of heartbeat on auscultation. Contact vet advisors for help.

All animals, either found dead or having been euthanized, must undergo a full post-mortem examination with collection of samples for culture, histopathology and PCR testing, and in certain cases, storage. A comprehensive protocol (Elephant Necropsy Protocol EEP) is available in Appendix VIII or online at **EAZA Elephant TAG SharePoint**. Post-mortem results must be submitted to the relevant EEP coordinator and vet advisors. If practically possible, research samples should be taken and stored which include frozen tissues and blood, and sections in formalin. For more details contact EAZA Biobank (**biobank@eaza.net**).

2.8 SPECIFIC PROBLEMS

2.8.1 FOOT CARE

Anatomy

The distal limb of an elephant is composed of the weight bearing structures and the surrounding skin. In the African and the Asian elephant, five bony digits are formed in the front as well as the hind foot (Fig. 29). Other anatomical features of the elephant foot include the cuticle, nail wall, interdigital space, nail sole and foot pad (Fig. 30). In the Asian elephant, the distal phalanx of digits 1 to 5, and digits 2 to 5 of the front and hind feet respectively, is protected by formed toenails. In contrast, in the African species, toe nails are formed at the end of digits 2 to 5 of the front and 2 to 4 of the hind foot. Formation and number of toe nails may vary between sub-species and even individually (Csuti *et al.*, 2001). The skin on the elephant´s foot is variably modified depending on the anatomical region. In the interdigital space as well as proximally adjacent to the pad, there is a dermal structure similar to that of the other body regions. The toe nails represent skin modified in the form of a horn capsule and the pad is built up out of thick horny layers (Benz, 2005).

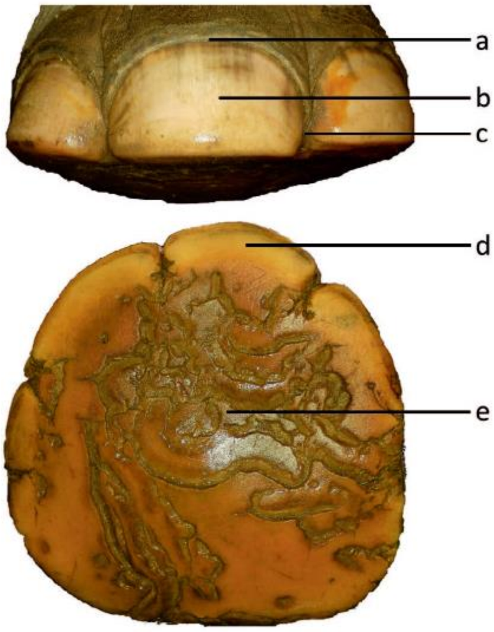

Figure 29 (left**)** Five bony digits are formed in each elephant foot.

Figure 30 (right) Definition of anatomical terms in the elephant foot: *a) cuticle, b) nail wall, c) interdigital space, d) nail sole, e) pad* (taken from Wendler *et al*., 2019a).

The cushion is a unique structure in an elephant´s foot (Weissengruber *et al.,* 2006). It is composed of strands of fibrous connective tissue forming compartments which are filled with adipose tissue (Fig. 31) (Weissengruber *et al*., 2006). The compartment of the cushion is further supported by a 6th digital ray, named prepollux or prehallux, of the fore and hind limbs respectively (Fig. 32). This cartilaginous rod runs from medio-proximal to latero-distal without touching the ground directly (Fig. 32) (Hutchinson *et al*., 2011; Weissengruber *et al.*, 2006).

Figure 31 Sagittal section of a male African elephant´s hind foot showing the supporting bony structures as well as the digital cushion (during the preparation process the adipose tissue dissolved and left the white areas between the strands of connective tissue) (© J. Völlm).

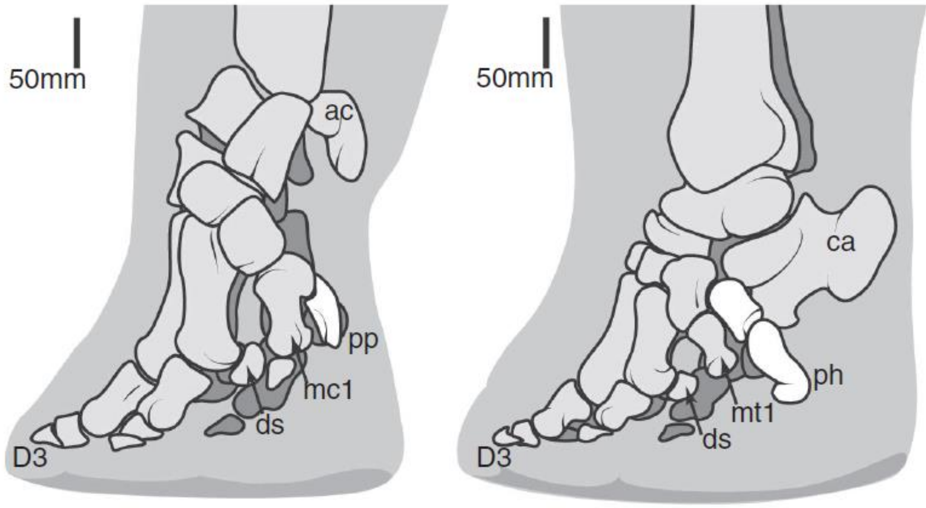

Figure 32 Medial aspects of the right feet of an elephant. *pp* indicates prepollux, *ph* prehallux. Both are running from medio-proximal to latero-distal (taken from Hutchinson *et al*., 2011).

Although elephant skin is generally lacking sweat glands, such structures have been detected between the toe nails and along the cuticles in the Asian elephant (Lamps *et al.*, 2001). These glands secrete a clear fluid, and their epithelium contains hormone receptors (Lamps *et al.*, 2004). The latter might indicate a relationship with chemical communication although the glands' function remains unclear (Lamps *et al.*, 2004).

Physiology

According to the anatomical basis of their feet, elephants are considered digitigrade (walks on toes) in their front and semiplantigrade (walks with toes and metatarsals partially on the ground) in their hind legs (Mikota *et al.*, 1994). When walking, the elephant´s hind foot treads into the print of the fore foot (Benz, 2005). Although elephants are not able to trot or gallop due to the direction of their limb bones, they can reach high speeds of up to 25 km/h (Benz, 2005; Hutchinson *et al.*, 2003). The digital cushion has a shock-absorbing function and allows elephants to walk silently even in forests (Weissengruber *et al.*, 2006). Furthermore, the elasticity of the cushion allows storage and return of strain energy during walking (Weissengruber *et al*., 2006). An obvious indicator of the elasticity of the cushion and subsequently of the entire foot, is the circumference change visible every time an elephant puts weight on it. In adult elephants, weight-bearing foot circumference increases in a range of 4-10% compared to the non-weight-bearing circumference (unpublished data Wendler; Csuti *et al.*, 2001). A further benefit of the cushions' elasticity might be its effect on the surrounding blood vessels in the way of a venous pump (Panagiotopoulou *et al.*, 2016). In theory, the latter will be effective during intermittent loading and unloading of the foot as it is the case during walking.

Apart from protecting the distal phalanx, toenails have variable functions. They facilitate climbing (Fig. 33) as well as digging and are used in the preparation of food (debarking branches, freeing grass roots from dirt). Investigating captive elephants, Benz (2005) determined the growth rate of the horn wall to be around 6-7mm/28 days with significant variation between front vs. hind feet and the African vs. Asian species.

Figure 33 Facilitating climbing is one function of the toenails.

Several studies investigated the locomotor mechanics in elephants including foot pressure measurements (Genin *et al*., 2010; Panagiotopoulou *et al*., 2016; Panagiotopoulou *et al*., 2012; Ren *et al*., 2008). The latter demonstrated highest concentration of peak pressures in the lateral digits in both elephant species. Throughout the stance phase, the center of pressure runs through the median of the sole, from the palmar/plantar to the dorsal part of the central toe (Fig. 34) (Panagiotopoulou *et al*., 2016).

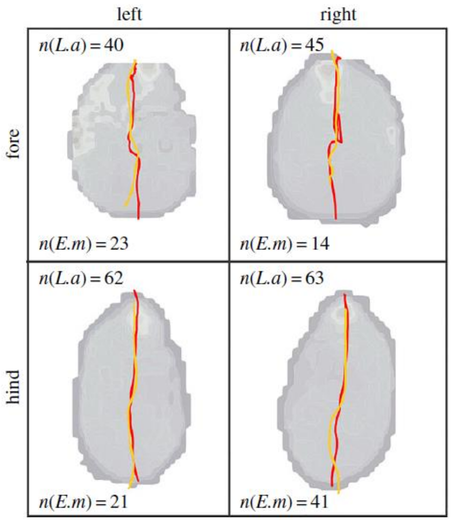

Figure 34 Mean center of pressure patterns in the African (red line) and Asian elephant (yellow line) during the stance phase of walking (taken from Panagiotopoulou *et al*., 2016).

Anatomical studies of the elephant's pad revealed the existence of sensory cells, so-called mechanoreceptors, which might be capable of detecting seismic properties of low frequency vocalizations (O´Connell *et al*., 1999; Weissengruber *et al*., 2006). If this assumption is correct, the elephant´s foot may play a relevant role in long-distance communication.

Management and Care

Foot health is critical for the physical health of the heaviest terrestrial mammal on earth. Although elephant owners are aware of this aspect, and our understanding of the underlying anatomy and physiology continues to increase significantly, foot health issues do still represent one of the most frequent health concerns in zoo elephants (Edwards *et al*., 2019; Wendler *et al*., 2019a). Despite this fact, there is only one standard textbook on elephant foot care, which has been published nearly 20 years ago (Csuti *et al*., 2001). Therefore, knowledge and skills concerning elephant foot care are mostly developed within elephant keeper teams of zoological institutions, and different husbandry conditions required different approaches, so that there might not be one single way in doing proper foot care. In this section, we present some general recommendations which should be adapted to the specific case and individual elephant.

The precondition for checking as well as trimming elephant feet is having an infrastructure allowing appropriate access to each elephant foot. This includes the training of the elephant to allow inspection of each foot, at any time and in various positions. The latter should be comfortable for the elephant but also for the staff doing the physically demanding trimming. Naturally, safety precautions need to be ensured as well. In protected contact, appropriate height, size and construction of the openings in the training wall are critical. Having adaptable openings is considered highly beneficial because they allow flexible adaptation to individual elephant sizes and joint mobility (Fig. 35). The latter might be of increased relevance in geriatric elephants or individuals suffering from degenerative joint disease. In such individuals, it is recommended to conduct foot care in short bouts to avoid staying in unnatural positions for extended periods.

Figure 35 Foot care opening in the training wall in a protected contact system. Note the adaptable crossbars.

With respect to the often protracted and elaborate treatment of foot disorders, a preventive approach is considered to be of utmost importance. Hence, it is strongly recommend to conduct daily monitoring and physical checks of every part of the foot including toe nails, interdigital spaces, cuticles, and foot pad. Proper inspection is only feasible after washing the feet and under good lighting conditions. Daily monitoring should include observation of standing behavior and gait. Elephants might suffer from severe foot lesions without showing distinct lameness (Lewis *et al.*, 2010). Often, alterations in the posture while standing can be seen earlier in the disease process rather than changes in the gait pattern (Fig. 36).

Figure 36 This geriatric female Asian elephant suffers from painful lesions in both front feet and struggles to shift its weight towards the hind quarter.

Considering the fact that most captive elephants use their feet less compared to their free-ranging counterparts and are provided a constant, high-quality diet, foot disorders might be due to insufficient wear rather than overgrowth. Nevertheless, the result remains the same and requires regular trimming of the feet. There is no generally recommendable interval for foot trims rather, it is recommended to conduct demand-based interventions in accordance with daily monitoring. If housing conditions facilitate walking behavior and natural use of the feet in foraging as well as digging behaviors, intervals of foot trims might be extended with only minimal interventions being necessary. Moreover, soft substrates have been shown repeatedly to reduce the risk for foot disorders in zoo elephants and are strongly recommended when (re-)constructing elephant exhibits (Miller *et al.*, 2016; Wendler *et al*., 2019b). We consider prevention the most favorable approach and recommend working on the corresponding husbandry conditions.

Practical considerations regarding foot care

To keep foot structures in a favorable condition or to correct alterations, a variety of tools can be used. These are hoof knives of variable shapes and sizes, rasps and files. In cases where high precision is required, it might be helpful to use wood carving tools as well. Independently of the chosen instrument, its sharpness is most relevant. Hence, it is strongly recommended to check this before starting the trimming. More recently, several elephant holders started to use electric grinders for foot care. Although elephants seem to tolerate these instruments if accommodated to the noise and vibration, we recommend caution in considering their use. While they are highly beneficial in relieving the physical workload and speeding up trimming time, potential damage through heat production might be underestimated. Additionally, it is more difficult to estimate how deep the trimming is done with these tools, and the risk of trimming too much might be increased. If these tools are applied, we strongly recommend using appropriate grinding disks and low rotational frequencies. It should be kept in mind that electric grinders are usually constructed for the trimming of cattle hooves. In the latter, the hoof horn is much harder than in elephants. Moreover, hoof horn quality and hardness in elephants are known to vary significantly inter- as well as intra-individually. Hence, we recommend using manually controlled devices for foot trimming in elephants. Further research might provide more evidence-based recommendations in this subject in the future.

Soaking and bathing of affected feet with and without antiseptics is often recommended. Although this might support the cleaning, we wish to add the following aspects for consideration. In sole abscesses with exposed dermis, extended soaking might have the drawback of transferring the microorganisms deeper into the tissue due to the dermal papillae acting similar to a wick. Hence, we recommend keeping soaking bouts short. In solar horn and pad lesions, soaking might only be effective if the applied container is adapted to allow circulation of the solution below the foot. If these aspects are considered, soaking may be helpful and have a softening effect on the nails, which may facilitate subsequent trimming. Soaking can never replace a therapeutic pedicure.

Agent	Indication	Comment
Wound cleansers		
Commercial soaps	Initial preparation and decontamination of heavily contaminated wounds.	Toxic to fibroblasts and should not be used in advanced wound cleansing.
Commercial wound cleansers	Initial preparation and decontamination of moderately contaminated wounds.	Neutral pH solutions should be preferred.
Tap water	Decontamination of wounds and early lavage.	Acceptable for early lavage.
Sterile infusion fluids (e.g. 0.9% saline, lactated ringer´s solution)	Lavage of decontaminated wounds.	Non-toxic to fibroblasts. No irritant effect.
Antiseptics		
Povidone Iodine	Irrigation of abscesses and wounds.	Has a broad antimicrobial activity and 4-6 hours of residual action. Inactivated by organic material and blood.
Chlorhexidine	Reduces antimicrobial contamination of wounds.	Has a broad antimicrobial activity. Not as irritating to healing tissues compared to substances.
Hydrogen Peroxide	Deep wounds, abscesses with anaerobic bacteria, initial treatment only.	Has a narrow antimicrobial spectrum, especially anaerobic bacteria and is not recommended for wound management.
Topical antibiotics (should be used based on culture of infected wounds)		
Silver sulfadiazine	Infected wounds with susceptible pathogens.	Broad antimicrobial action including *Pseudomonas sp.* and fungi.
Nitrofurazone	Infected wounds with susceptible pathogens.	Good antimicrobial effect but not against *Pseudomonas sp.*
Gentamycin sulfate	Infected wounds with susceptible pathogens.	Narrow spectrum of antimicrobial action but effective against

		Pseudomonas sp. and gram-negative pathogens.
Antibiotic spray ("blue spray")	-	Poor penetration and mainly placebo effect. Not recommended.
Topical herbal and alternative therapies		
Aloe vera	Chronic wounds with low healing activity.	Stimulates local immune response and wound healing. Shows some antimicrobial properties. Use in elephants not reported so far.
Medicinal honey	Wounds with low regeneration activity.	Broad spectrum antimicrobial and anti-inflammatory properties. The high osmotic value of honey promotes drainage of the wound. Stimulates new tissue growth. Anecdotal use in elephants with promising results.
Low level laser therapy (LLLT)	Chronic wounds with low healing activity.	LLLT has been shown to increase wound healing in horses. Use in elephants anecdotal but not reported evidence-based so far.

Table 10 Commonly used agents for foot baths and wound lavage in elephants (modified from *BIAZA Management Guidelines for the welfare of elephants (2010)*). Note that further agents may be required depending on the specific lesion.

To avoid contamination and irritation with dirt, the application of a protective boot has been proposed (Csuti *et al.,* 2001). Based on our experience we do not recommend this approach, since humidity, body warmth and low oxygen concentration inside the boot may provide an ideal substrate for pathogenic microorganisms. We consider it more beneficial for the healing process to allow proper drainage of secretions and air contact to let the lesion dry. In order to reduce contamination with dirt, we recommend keeping affected elephants temporarily on a relatively clean substrate (rubber, concrete). In such cases it will be highly beneficial to have a concrete floored box available, despite the general advantages of soft substrates.

In cases of more severe disorders, further diagnostics by radiographs are strongly recommended (Hittmair & Vielgrader, 2000; Mumby *et al*., 2013). This method presents an effective tool for determination and monitoring of affected bony structures, although individual anatomic variation should be taken into account (Regnault *et al*., 2017).

If restricted to the distal limb, abscesses and infections are usually treated locally with antiseptics and antibiotics. Whether or not systemically administered antibiotics can reach appropriate acting levels in the dermal structures of the foot is questionable. In severe cases, repeated intravenous perfusion has been reported as a very effective method of administration, even in cases of osteitis (Dutton *et a*l., 2017; Ollivet-Courtois *et al*., 2003).

Conformational abnormalities can cause an uneven pressure distribution pattern in an elephant´s foot with subsequent chronic disorders. Although corrective trimming might allow symptomatic treatment, a correction of the conformation might be needed to improve the underlying cause. Although promising results have been recently reported with glue-on shoes, further research in this method is needed to evaluate its feasibility (Johnson *et al*., 2018).

Currently, there is no evidence on the beneficial effect of feed supplementation of Biotin (vitamin B_7) (Wendler *et al*., 2019b). Although a positive effect on hoof quality has been shown in horses and diet supplementation leads to elevated serum biotin levels in elephants, further research is needed to determine its effect on foot health (Benz, 2005; Wendler *et al*., 2019b).

Accurate documentation of each individual elephant´s foot condition is strongly recommended. Doing so will enable reevaluation and confirmation of progress over extended treatment periods. Example documentation forms have been provided by Benz (2005) (Fig. 37). Additionally a more objective approach to scoring and monitoring of foot health has been developed recently (Ertl et al., in press). The authors of the latter provide an Excel®-sheet allowing the automatic calculation of the foot score (Fig. 38). The Excel sheet will be available on the EAZA Elephant TAG document page or by request from Dr. med. vet. Paulin Wendler (paulin.wendler@web.de).

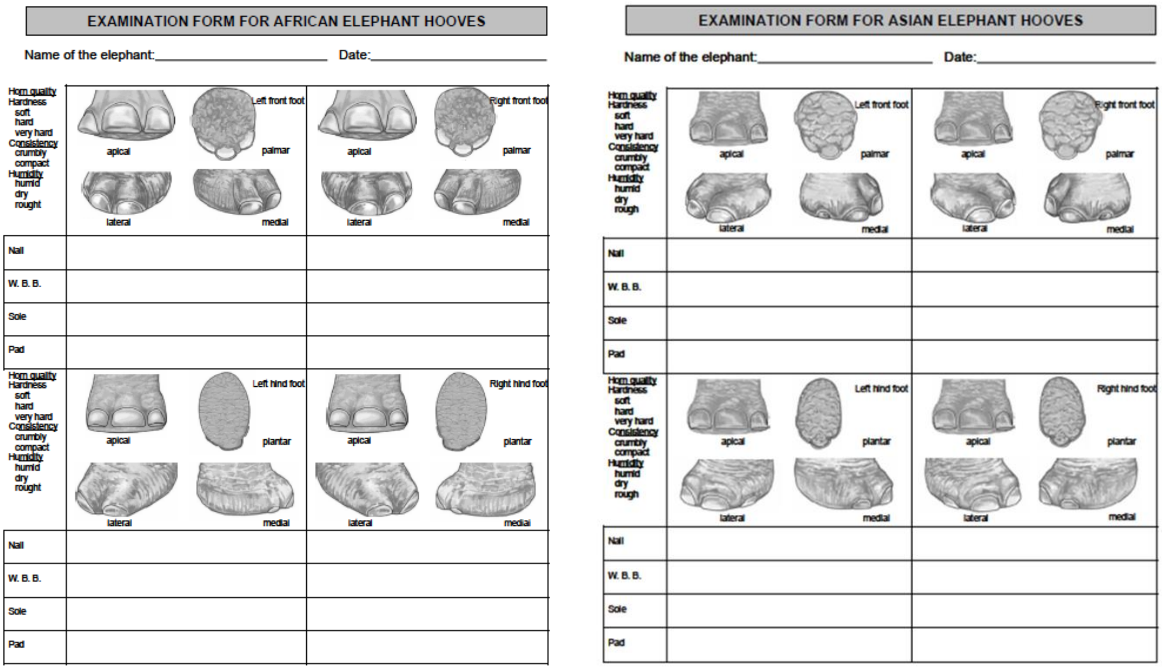

Figure 37 Example species-specific documentation forms provided by Benz (2005).

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	Paulin Wendler, University of Zurich. For questions and suggestions please send me an email to paulin.wendler@web.de.														
2	**Institution:**														
3	**Name of the elephant:**						Type X (as capital letter) if the respective pathology occurs, the scores will automatically be calculated.								
4	**Date:**														
5															
6				mild		moderate				severe					
7			Normal	Minor nail crack	Attached cuticles	Solar horn defect	Major nail crack	Fluid pockets	Soft pad	Purulent nail	Purulent pad	Altered nail tissue	Apical lesion	Value	Squared value
8	left front	N1												0	0
9		N2												0	0
10		N3												0	0
11		N4												0	0
12		N5												0	0
13		Pad												0	0
14	right front	N1												0	0
15		N2												0	0
16		N3												0	0
17		N4												0	0
18		N5												0	0
19		Pad												0	0
20	left hind	N2												0	0
21		N3												0	0
22		N4												0	0
23		N5												0	0
24		Pad												0	0
25	right hind	N2												0	0
26		N3												0	0
27		N4												0	0
28		N5												0	0
29		Pad												0	0
30															
31													**ParSev Score:**		**0**
32													(Sum of squared values)		
33															

Figure 38 Excel®-sheet to calculate an objective foot score (ParSev Score) according to Ertl et al. (in press).

Ideal condition of foot structures

In the following section examples of the ideal condition of each foot structure are provided in order to allow comparison while doing daily checks.

Interdigital space

Figure 39 Front foot of a female African elephant. The skin in the interdigital space should be dry and without any irritation. Ideally, the space between the nails should be of at least one finger´s width.

Cuticles

Figure 40 Well-conditioned cuticles in the front foot of a female African elephant. The cuticles present the dorsal junction between the skin and the nail capsule. Ideally, the cuticle passes smoothly into the nail without any swelling, thickening or feathering. Due to the aforementioned sweat glands the area of the cuticles might show some humidity, particularly on hot days (Lamps *et al*., 2001).

Nails/Sole

Figure 41 Well-conditioned nail wall in the hind foot of an Asian elephant (©Pauline Wendler/Nicolas Ertl).

Figure 42 Well-conditioned sole region of the nail in the front foot of an Asian elephant (©Pauline Wendler/Nicolas Ertl).

Based on the anatomy and physiology discussed above, it becomes obvious that the elephant's toenails have no weight bearing function. Hence, when the elephant is standing on a hard and even surface, there should ideally be no pressure on the nails. The shape of each nail may differ individually as well as the thickness and hardness of the horn capsule.

Pad

Figure 43 Ideal pad condition in the front (left) and hind foot (right) of an Asian elephant.
The profile of an ideal well-worn pad looks similar to the structure of a car tire. This means that there may be several grooves, which ensure grip on various surfaces. These shallow grooves should be differentiated from fissures. Horn quality of the pad is known to vary individually as well as the shade/hue of the pad tissue (left picture ©Pauline Wendler/Nicolas Ertl).

Foot structure conditions requiring treatment

Similar to the example pictures for the ideal condition of the foot structures, examples of foot related disorders are provided below, along with a shorthand proposal for treatment. Further discussion of the specific pathologies and their treatment is not within the scope of this section, but corresponding information can be found in the literature (Csuti *et al*., 2001; Landolfi & Terrell, 2018).

Interdigital space

Figure 44 Too narrow interdigital space in the front (left picture) and hind foot of a female Asian elephant.
Note that the corresponding nails are nearly rubbing.
Proposal for treatment: *Nail trimming to bring them into natural shape and widen the interdigital space.*

Cuticles

Figure 45 Feathering of the cuticle (and severely overgrown nail) in the hind foot of a female African elephant (left) and the hind foot (right) of a female Asian elephant.
Proposal for treatment: *Trimming of the cuticles, rasping/filing the nail surface to create a smooth transition between the cuticle and the nail wall.*

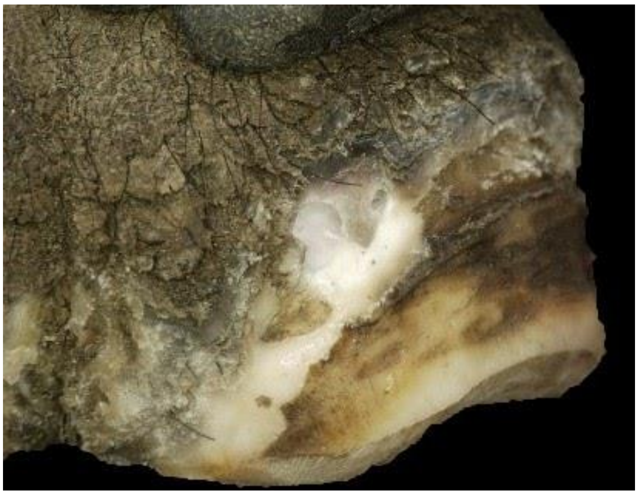

Figure 46 Fluid pockets in the overgrown cuticle (©P. Wendler/N. Ertl).
Proposal for treatment: *Trimming of the cuticles to create a smooth transition to the nail wall.*

Figure 47 Significant thickening and overgrowth of the cuticles in the front foot of a female Asian elephant.
Proposal for treatment: *Trimming the cuticles.*

Nails/Sole

Figure 48 Overgrown nails with horn rings in the front foot of a female Asian elephant.
Proposal for treatment: *Trimming of the nails to bring them in a natural shape.*

Figure 49 Severely overgrown nails in the front foot of a female African elephant.
Proposal for treatment: *Trimming of the nails to bring them in a natural shape. Examination of the leg conformation which might cause the uneven nail growth.*

Figure 50 Vertical and horizontal nail crack in the front feet of a female Asian elephant.
Proposal for treatment: *Trimming of the nails, keeping the cracks clean, determining and eliminating the underlying cause of crack development.*

Figure 51 Nail abscess with opening in the sole region of the nail in the front foot of a female Asian elephant
Proposal for treatment: *Alternatingly trimming and letting the superficial tissue layers atrophy until healthy granulation tissue is present. Undetermined and hypergranulation tissue needs to be removed gradually.*
Keep the lesion clean.

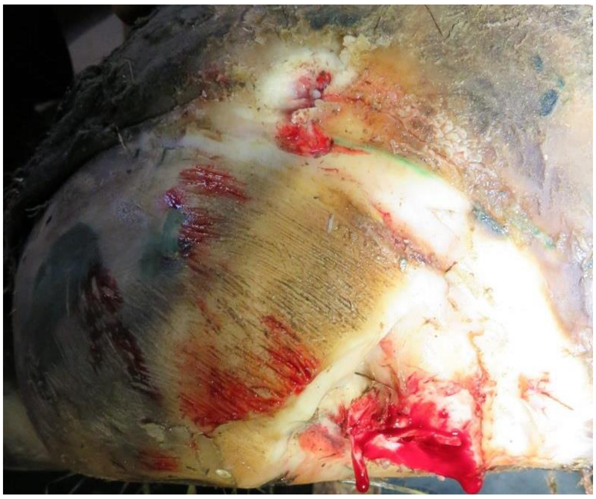

Figure 52 Chronic abscess with openings in the sole as well as the coronary region in the front foot of a female Asian elephant.
Proposal for treatment: *Alternatingly trimming and letting the superficial tissue layers atrophy until healthy granulation tissue is present. Undetermined and hypergranulation tissue needs to be removed gradually. Keep the lesion clean. Radiographs to determine whether bone is affected. Intravenous perfusion with antibiotics should be taken into consideration.*

Figure 53 Multiple abscesses (indicated by yellow arrows) in the nails of the front feet of a female Asian elephant. Note the protruding granulation tissue and the relieving behavior (alternating lifting of both feet). ***Proposal for treatment:*** *Alternatingly trimming and letting the superficial tissue layers atrophy until healthy granulation tissue is present. Undetermined and hypergranulation tissue needs to be removed gradually. Keep the lesion clean. Radiographs to determine whether bone is affected. Intravenous perfusion with antibiotics should be taken into consideration.*

Important note regarding nail lesions:

With respect to the growth rate of the horn wall of approximately 7 mm/month, the repair of a defect in the coronary region of the nail will take almost one year if a horn wall height of 7.5 cm is assumed (Benz, 2005).

Pad

Figure 54 Extended defect in the pad of a female Asian elephant´s hind foot (left picture) and overgrown pad in the front foot of a female Asian elephant.
Proposal for treatment: *Trimming of the pad. Undetermined tissue layers need to be removed gradually. Keep lesions clean.*

Photo credit in this chapter: where not indicated differently copyright Christian Schiffmann.

2.8.2 GERIATRIC CARE

With better husbandry techniques and improved veterinary knowledge, elephants live comfortably to a greater age in captivity. Older elephants present new challenges in human care, from joint problems to variation in temperament and are often good at masking a deterioration in health. Therefore, health and welfare monitoring of older animals is vital and may be required more frequently than in younger adult animals. Geriatric care can sometimes involve specialised procedures and techniques, which may require specialised staff and elephant training. Currently the older animals in the population are considered "legacy" animals and may suffer from specific problems which could have arisen due to previous poor management situations. These may exacerbate geriatric issues.
The following aspects should be considered when caring for geriatric elephants:

Diet

Due to a reduction of grinding surface or even loss of molar teeth, chewing efficacy can be significantly reduced. This can result in constipation caused by increased faecal fibre length. Colic symptoms (see section on colic above) might be observed in a constipated elephant.
Prevention: Regular inspection and documentation of molar status and faecal quality/size/shape. The structure of the diet should be adapted in response to the latter. This can be acheived by the provision of chopped hay, soft food and/or formulations for geriatric horses. Moreover, the administration of stool-softening supplements (e.g. flax seeds, bran or plant oil) is recommendable. Maintaining an appropriate level of fibre content as well as gradual changes in the diet is critical (see **section 2.2 Feeding**).
Furthermore, the reduced chewing efficacy may lead to a loss of weight/decline in body condition due to decreased energy absorption. This can be addressed by the provision of an more energy-rich diet (e.g. increased amount of lucerne hay) and inclusion of supplements (rice, concentrates). To administer the specific diet to the specific individual, a temporary separation from the group should be taken into consideration.

Activity

Degenerative joint disease is common in geriatric elephants and may lead to a reduction in mobility as well as activity. **Prevention:** Through a sophisticated feeding and training program the activity level of an older individual can be increased and mobility maintained to a certain degree. Additionally, training sessions will support mental stimulation even in geriatric elephants.

Resting

Due to joint stiffness and/or pain, geriatric elephants are known to change their resting behaviour (Schiffmann *et al*. 2018). **Prevention:** Therefore, the provision of appropriate structures for lying (e.g.

sand piles/slopes) as well as leaning rest and soft substrates in general are highly recommended (Schiffmann *et al*. 2018). Resting behaviour should be recorded and documented on a daily basis.

Quality of life assessment

We strongly recommend the regular assessment of quality of life in geriatric elephants by use of a corresponding form (see Appendix IX). The assessment should be conducted by all staff involved and should be implemented at an early stage. Assessment of outcomes provide a rather objective evaluation of quality of life and must be documented.

2.8.3 SLEEP

Sleep should be monitored routinely with time spent lying down, length of sleep and side preference recorded. All elephants should continue to sleep in a recumbent position regardless of their age. If elephants routinely prefer to sleep on one side, sores may appear on the hip, elbow, shoulder and temporal areas; these must be treated in accordance with veterinary advice. There should be ample opportunities for animals to rest through leaning or lying down. If animals are not routinely lying down, veterinary advice should be sought. Sand pillows can be used to aid animals in getting up. Large tree stumps and logs can be used to encourage elephants to lean or rest on (example given in Schiffmann *et al*. 2018b).

Figure 55 Sand pile for sleeping ©Christian Schiffmann

2.9 RECOMMENDED RESEARCH

Research is one of the principal indirect conservation roles of elephants in held in EAZA zoos. Data obtained from *ex situ* zoo-housed elephants can provide valuable information for *in situ* conservation efforts and help advance knowledge of elephant biology and management as well. Well planned, well run *ex situ* breeding programmes can provide much needed information on a variety of areas to aid in answering important questions that directly impact overall global conservation priorities. These include, but are not limited to, health, behaviour, reproduction and nutrition.

Zoological records maintained in suitable formats (e.g. ZIMS) are a valuable source of data on captive elephants, with historic information providing longitudinal reference data. Utilising records data from multiple institutions can help to alleviate the issues of small sample sizes (often a problem with zoo research). Necropsy information can provide data that are otherwise unobtainable and research utilising this data should also be considered.

Research on zoo elephants informs evidence-based husbandry, encouraging best practice on both an individual collection and population level, and importantly, demonstrates compliance with key legislation. Additionally, information gained from research on captive elephants can aid wild counterparts and captive elephants in range state countries.

Many of the research areas can now be aided using appropriate technologies such as GPS equipment to monitor behaviour and enclosure use (Leighty *et al.*, 2010) and thermal imaging equipment as a diagnostic health tool (Avni-Magen *et al.*, 2017). This application of technology for zoo research will continue to develop and can be a viable way for *ex situ* elephant populations to contribute to field conservation initiatives, for example by validating use of new technologies in ways that cannot be undertaken in the wild.

Modern conservation applies the One Plan Approach (Byers *et al.*, 2013), whereby all stakeholders involved with species populations (whether inside or outside of natural ranges) are involved in management strategies and conservation actions. This approach is enhanced by contributions from *ex situ* captive populations.

All collections holding elephants should undertake or engage with research supported directly or indirectly by TAG. Zoos are also encouraged to participate in IUCN Species Specialist Group-supported research projects where possible.

For the update on current research carried out in EAZA zoos, see the PowerPoint presentation of Dr. Christian Schiffmann (“Current research in European zoo elephants and future direction“) presented at the EAZA Annual Conference in Valencia in 2019 or see “Research publications based on Asian and African elephant ex situ populations“ which are both available on **EAZA Elephant TAG SharePoint**.

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APPENDIX I

To breed or not to breed …
– monitoring female reproductive status to enhance or avoid pregnancy in elephants -

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Background

Both, African and Asian elephants are increasingly threatenend in the wild. Populations grow well in undisturbed areas but African elephants are still poached for ivory and Asian elephants are constantly driven out of their natural habitat and killed in human-elephant conflicts. At the same time breeding success in captivity is low in most countries of the world. In Europe, long-term efforts have been made to build up self-sustaining populations. The African elephant is currently still in a vulnerable situation but the Asian elephant has recently reproduced so well that for some animals breeding stops are recommended. **Irrespective of whether breeding is wanted or not, monitoring of female reproductive status is the key for an effective breeding management and can be used to either help to enhance or to avoid pregnancy.**

Methods

Monitoring of reproductive status is best performed via longitudinal measurements of hormones. Hormone analysis provides an indirect method to determine the function of the ovaries and to detect the time of ovulation. Ovarian hormones can be detected in blood, urine, feces and saliva. Given the long cycle of 14 - 15 weeks in elephants, only one sample per week for an initial period of 6 months is required to obtain reliable results. If a cow is diagnosed to be cyclic during this period, it can then be decided if sampling needs to be continued in order to have her mated or not.

Practical advice:
Whatever method you chose, you need to be sure about 4 things:

1. *You need to get samples from your elephants in **weekly intervals***
2. *You need to be sure that the values you get from your lab are **reliable and comparable***
3. *You need to know **what the values mean***
4. *You need a lab which provides **results within a week***

***WHY?** It does not help if you get a sheet with numbers and do not know what they say … and it is of no use to know that your elephant cow was in estrus 3 months ago! It is better to know when she WILL be in estrus again! For this you need frequent and quick results!*

Hormones

As in most other mammals, gestagens are also the best indicator for ovarian activity in elephants, with low levels during the follicle phase of the cycle and high levels after ovulation and thus during the luteal phase of the cycle. In contrast to most other mammals, however, progesterone levels in blood are extremely low in elephants! Most labs therefore fail to detect follicular phase values! As a result, blood results are not always reliable and data can vary substantially depending on the assay system in use! Hormone analysis in **blood** is generally possible in every hormone lab (hospitals, gynecologists, vet labs, other service labs). However, it is important to set up your own data base for your own animals with your "home lab " - which in the best case is close by because you need quick results – latest when it comes to a birth!

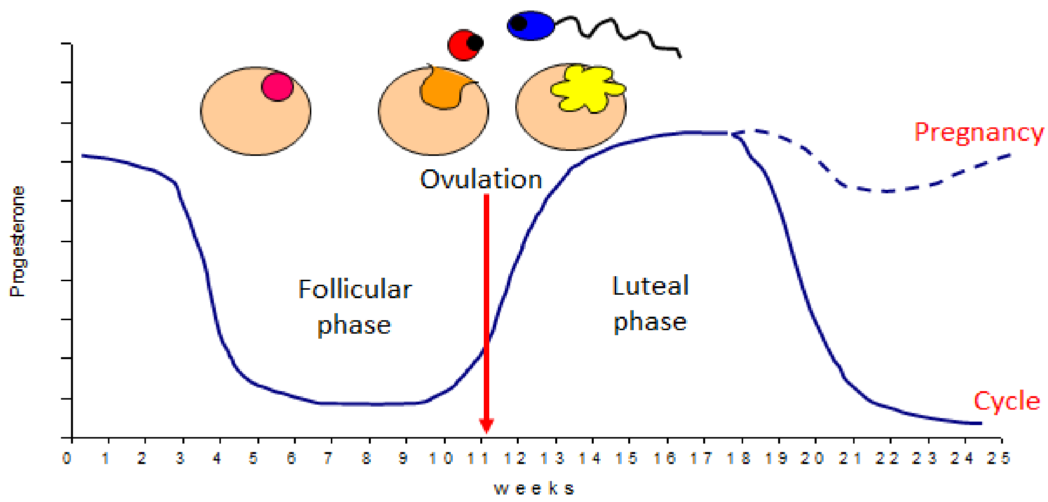
Urinary hormone analyses are performed in the Endocrinology Lab at the German Primate Centre in Goettingen, Germany. All facilities in Europe are welcome to send samples on a voluntary basis and assays are run every week. The service for the Asian elephants started in 1994 and that for African elephants in 1996, so comparative data are available for more than 20 years. It is important to note that in urine not progesterone itself but its metabolites are measured. These metabolites differ between the two elephant species. Whilst in Asian elephants pregnanetriol (P3) is the most abundant metabolite in urine, it is 5α-pregnane-3-ol-20–one (5α-P-3-OH) in African elephants. This means that different assays are needed for Asian and African elephants!

For **fecal** hormone measurements samples need to be send to the Endocrine Lab in Chester Zoo, United Kingdom. **Saliva** tests have not been validated accurately for either of the two elephant species.

Reproduction basics

The cycle of an elephant lasts 14 - 15 weeks, i.e. 3 - 4 month. Each cycle consists of two phases, the follicular and the luteal phase. The follicular phase lasts 4 - 6 weeks and is characterised by the growth of follicles in the ovary, one of which will ovulate and release the egg or oocyte for fertilisation. After ovulation, the ruptured follicle transforms into a corpus luteum which secretes progesterone in order to prepare the uterus for the possible implantation of an embryo. The cycle phase following ovulation, i.e. the luteal phase, lasts 8 – 10 weeks. If, for whatever reason, no conception occurs, a new cycle starts.

Since progesterone is secreted by the corpus luteum, levels are low during the follicular phase and high during the luteal phase. Ovulation occurs in the week between the last low and the first high value. A schematic overview of the ovarian changes during the cycle of an elephant is given in the figure below:



© A.-K. Oerke, 2003

Only matings around **ovulation** can be successful. Ovulation is behaviourally detectable as **estrus or heat** with cows offering themselves to the bull or bulls being interested in cows - and in the best case mating and guarding them. If no bull is present to advertise estrus of the cow, keepers might also notice changes of her vulva (swollen, red, extruded) or secretion of mucus from her vagina. Some keepers even report a different smell in estrus cows. Elephant females may also show different behaviour, trying to keep extremely close in direct contact and not responding to commands in protected contact.

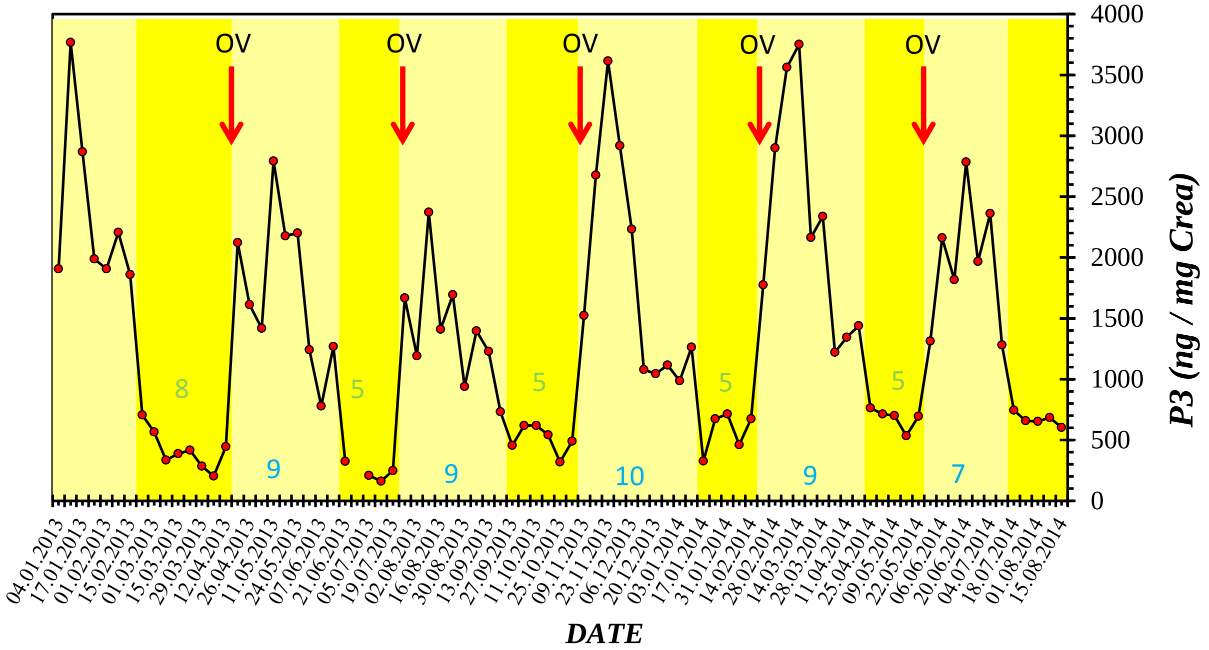
Prediction of estrus

The European Elephant Service in Goettingen provides weekly results and zoos sending samples regularly know EVERY week in which cycle stage their elephants are! This also means that estrus can be predicted! As soon as a low value indicates the start of a new follicular phase, the time of ovulation can be estimated based on the usual length of the follicular phase in this individual elephant. So it is possible to plan way in time for either placing the cow with the bull OR separating the bull from the cow in order to prevent mating!

Practical advice:

Elephant females are special in having a double peak of the luteinizing hormone LH which induces ovulation! LH is secreted for only one day (therefore peak!) and the first (non-ovulatory) peak occurs 3 weeks before the second (ovulatory) peak. Since only this second LH peak leads to ovulation only matings at this time can result in conception. However, not all bulls are able to discriminate between 1st and 2nd LH peak and mate at both times. You can use this behaviour as additional control to your cycle monitoring. Gestagens stay low after the 1st LH peak but rise as a result of ovulation after the 2nd LH peak!

An example for cycles of an elephant monitored by urinary hormone analysis is shown below:



Legend:
dark yellow = follicle phases (green numbers give duration in weeks)
bright yellow = luteal phases (blue numbers give duration in weeks)
OV = ovulation

Collection of urine samples

Urine can be collected in many different ways depending on keeping and housing system. In general only 2 ml urine are needed for the analysis. It is good to use plastic tubes that close well, best with skrew lid. Labelling of the sample is essential! Labels must be waterproof and show 1. name of animal and 2. date of collection. If the samples are not send straight (no cooling needed if they arrive within 2 days), they need to be frozen soon after collection.

It is possible to collect urine samples in all keeping systems: direct, protected or no contact! In fact, hormone analysis in urine samples was originally invented as a non-invasive method that needs no contact to the elephant. Therefore it is strange when zoos claim that they can not get samples from their elephants because they keep them in protected contact! Everything is possible and each zoo has its own method and tricks. One facility with protected contact collects weekly samples from 9 females EVERY week on the same day, including a 4 year old calf. For this, however, the urine collection must be part of the training routine!

In direct contact elephant females are usually trained with special commands to provide a sample. The same can be done in protected contact. The difficulty is to get access to the sample! Zoos have invented all kind of methods, from females standing parallel and in reach through the training bars, to telescope sticks with little pots to hold under the animal. Even paper towel is used to soak the urine which is then held over the tube to let the urine dribble in.

Alternatively, and in case of no contact, the urine can be collected from the ground - but it is important that the sample is not diluted with water from either washing the elephant or cleaning of the stable. If possible, contamination with faeces should be avoided An elephant can, for example, be locked in its box until it urinates. The provision of urine must initially be connected with a special command and followed by a reward. Once the elephant has left its box, the sample can be taken from the ground. If you are lucky the floor has a gradient and the urine comes running to you! You can also use the "Cabarceno-method" and drill little holes in the ground where the urine collects and you pick it up with a syringe later!

If urine collection is part of the training, elephant eventually start to urinate when they see you coming in with a tube and a reward. **Elephants are smart ☺**

In Europe 93 % of Asian elephants and 88 % of African elephants are cyclic. Young Asian elephant females start with ovarian cycles at the age of only 4 years whilst African elephants start with only 7 years! Cows that gave birth to a dead calf or that do not raise the young themselves resume cycle as early as 7 – 11 weeks after delivery, whilst mothers with live calves have their post-partum ovulation 50 – 55 weeks after birth.

Version 9/2019

APPENDIX II

Veterinary guidelines for reproduction-related management in captive female elephants

April 2020

Willem Schaftenaar, Thomas B. Hildebrandt

Elephant TAG veterinary advisors

The breeding process in elephants needs monitoring of several parameters in both males and females. The main tools for the determination of the estrous cycle, the evaluation of the genital tract in both sexes, the determination of the number of fetuses and finally the moment of parturition are hormonal assays (progesterone and in some cases LH assays) and ultrasonography.

The first part of this document briefly describes aspects of the estrous cycle and the monitoring tools that can be used in female elephants. The second part describes a protocol for veterinary intervention in elephant parturition. The third part describes the vaginal vestibulotomy procedure. This is not a static document. Colleagues are strongly encouraged to report any experience that may add useful information to these guidelines.

Calcium given as a drip to an Asian elephant during an interrupted parturition (second parturition)

Ruptured uterus of an Asian elephant after a failed parturition that lasted for more than 10 days. The calf had malformed legs.

Fetal membranes being removed by a group member after a successful parturition in a group.

I. Main aspects for monitoring and assisting female reproduction in elephants

Determination of the estrous cycle:

The estrous cycle can be divided in 2 phases, the luteal phase and the non-luteal or follicular phase. The luteal phase starts after ovulation and is characterized by elevated progesterone blood levels. The follicular phase starts when the progesterone blood concentration has dropped to what we call in this document the "base line" value. Most laboratory techniques fail to measure any progesterone in the blood during this phase in elephants. Some machines calculate these low concentrations rather than measure them exactly. Moreover, the progesterone concentrations that are provided by the different laboratories, vary greatly depending on the machine and technique used. *It is therefore very important to use the same technique and machine for monitoring the estrous cycle of an individual elephant.* During the follicular phase, approximately 18-20 days prior to ovulation, a 1-day LH-peak concentration can be distinguished, which may result in a temporary rise of progesterone. A second LH-peak occurs just prior to ovulation (see Fig. 1).

To monitor the estrous cycle in elephants gestagens should be determined in blood (progesterone), urine (pregnanetriol, Primate Center Göttingen, Germany) or faeces (progesterone, Chester Zoo, UK). Frequency of sampling should be once a week for blood and urine and 3 times per week for faeces.

Note: when using the urine test, one must be aware that the pregnanetriol concentration in the urine is always compared with creatinine. If the creatinine level is too low, a new sample should be submitted. Even when monitoring the cycle by urine-pregnanetriol, much effort should be made to train all elephants to allow blood sampling.

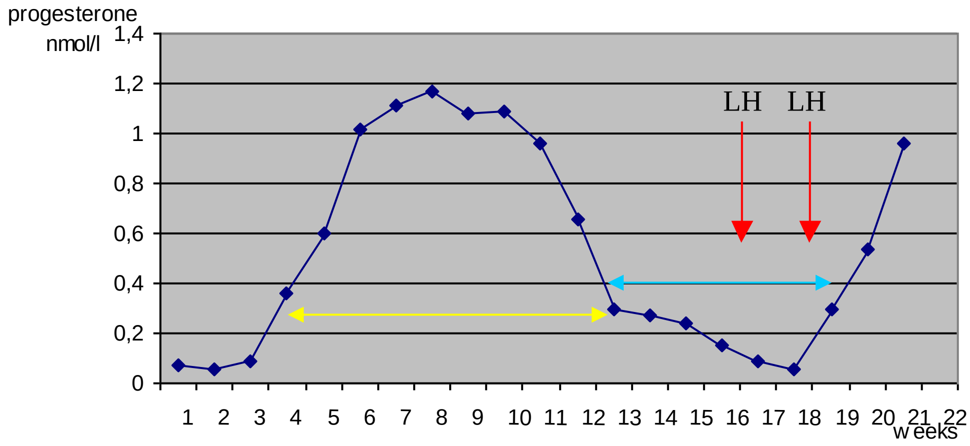


Figure 1 Progesterone blood concentration in nmol/l during an estrous cycle in an elephant. Note the 2 LH-peaks.

: luteal phase

: follicular phase

Measuring of blood progesterone in elephants:

Monitoring the progesterone concentration pattern in blood and/or pregnanetriol in urine is relevant for:

1. Determination of the right moment for mating
2. Pregnancy confirmation
3. To determine the moment that progesterone production has stopped at the end of pregnancy. In this case a quick result is needed in order to be able to act accurately. Therefore, measuring the progesterone levels at the end of pregnancy is preferred in blood rather than in urine, because the number of laboratories that can measure (urine) pregnanetriol unfortunately is limited to one (Primate Center in Göttingen, Germany).

When blood samples are submitted for the determination of blood progesterone, it is essential to be aware of the differences in the outcome. The most accurate results are obtained by using a Radio Immuno Assay (RIA). However, not many laboratories using this technique will be able to provide a daily service. Tests that are based on other immunological reactions (EIA/ELISA) are routinely used in human hospitals but they need to be validated for elephants before the results can be trusted. Figure 2 shows the differences in test results obtained by using different machines and different test units (1 nmol/l corresponds with approximately 0.32 ng/ml).

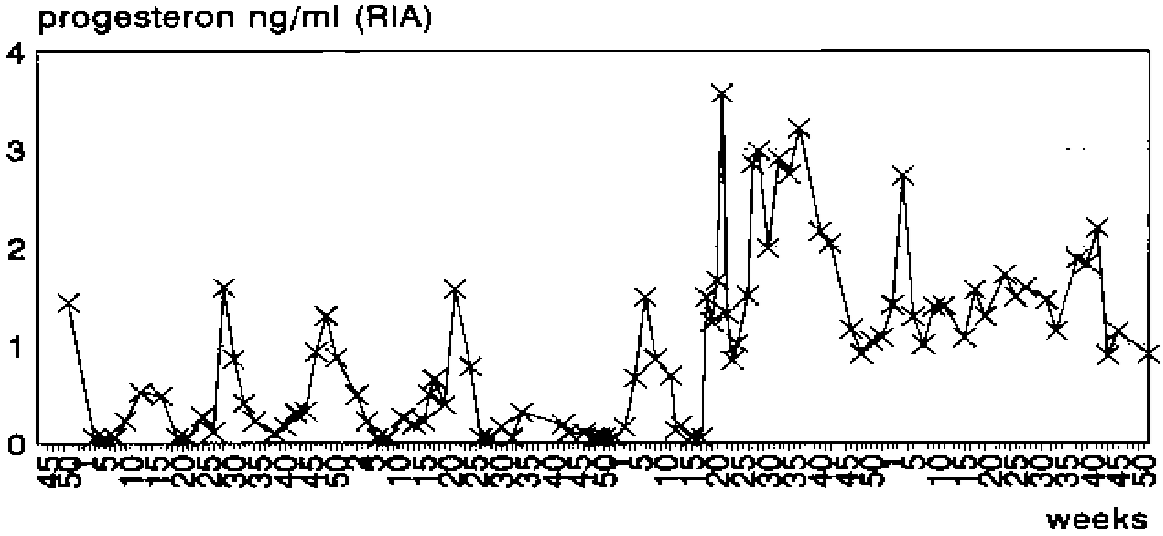


Figure 2a Blood progesterone concentration in an Asian elephant, determined by a RIA. Seven follicular phases, six complete luteal phases and the major part of the gestation period are clearly identifiable. Note that two of these luteal phases express themselves in relatively low progesterone concentrations. This lab measured the progesterone concentrations in ng/ml.

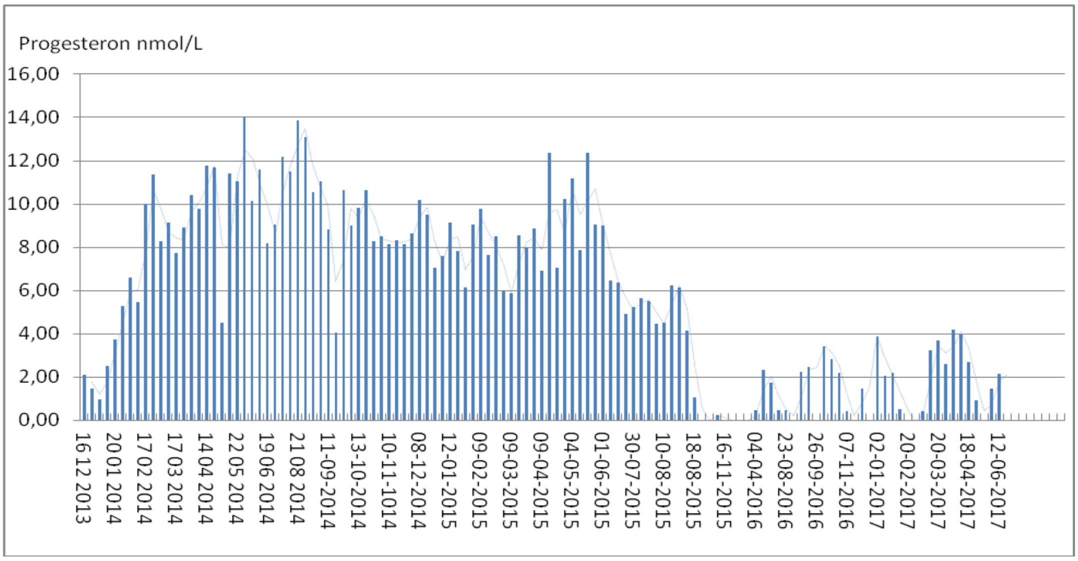


Figure 2b Blood progesterone concentration in an Asian elephant, determined by an ELISA (ADVIA Centaur CP – Siemens)). This graph clearly shows the terminal part of the gestation period with a sharp drop of progesterone (within 48 hours) to the baseline value specific for this elephant (18-08-2018). The calf was born within 48 hours after the progesterone concentration had dropped to base

Progesterone base line values:
The progesterone concentrations that are found during the non-luteal phase in elephants are often too low to be exactly measured by most techniques used currently. Some machines can calculate these values, rather than measure them. These low levels are found during the follicular phase, at the end of pregnancy and during the lactation and "menopause" anoestrous. The turning-point between luteal and non-luteal phase is what we call the "base line" value. The height of this base line value is rather arbitrary, but should be estimated for each individual animal. It should also be determined in relationship with the type of the assay used. Figure 2b and 2c make clear that the absolute values given by the laboratory may significantly differ between the machines that are used. This has already resulted in wrongly interpreted values: a progesterone concentration of 4 nmol/l was found in an a-term Asian elephant. As no blood samples were taken previously, this single value was considered a high concentration of progesterone. However, in reality it was a baseline value for this particular elephant measures on this particular machine. No action was taken as this signal of a stagnating calving process was not recognized. Consequently the calf in this case was born dead.

Preventing pathologic conditions:

- Elephants kept in zoos generally enter puberty at a much younger age than in the wild. This may have negative consequences for the reproductive organs. The occurrence of endometrial or ovarian cysts (cystic hyperplasia , frequently found in African and Asian elephants) or benign myometrium tumors (leiomyomas, exclusively found in older Asian females) may be the result of (normal) cyclic changes in the reproductive organs, if the cycle is not "rewarded" with pregnancy. To avoid these pathologic conditions, zoos should focus on a husbandry system that delays puberty as much as possible. The best way to achieve this condition is by raising young elephants (especially females) in intact families which may contribute to a natural way of social depression. The oestrous cycle should be monitored starting when the animal is around 3 years old. To reduce the risk of future pathological conditions in the reproductive organs, mating should take place when the young animal has gone through 2 years of normal oestrous cycles at maximum.
- For the same reasons as described above, the inter-calving period should be restricted to maximal7 years.

Oestrous period and pregnancy:
- The occurrence of mating is not an indicator for impending ovulation; some animals mate even during advanced pregnancy or outside the estrous period.
- Fertilization can only result from mating during estrous preceded by the pre-ovulatory (=second) LH-peak. Monitoring LH needs daily blood sampling for at least 10 days (A test kit is available presently: LH ELISA KIT, which can be ordered from Lenora Bruce, University of California Davis, Central Storehous Receiving, California and Larue, Davis CA 95616 USA, phone

+1-530-752-0663)

- The fertile period is restricted to the time around the second LH-surge, which occurs immediately prior to the rise in progesterone. For the prediction of the next ovulation, follicular phase length has to be determined and therefore it is important to know the moment of the preceding fall of progesterone/pregnanetriol. For reliable accuracy, weekly samples throughout the cycles have good predictive value.
- LH monitoring daily during the non-luteal phase is necessary for artificial insemination programs. Determination of the post-luteal phase (=first) LH-peak can help predict the first opportunity for (natural) breeding, as the interval between both LH-peaks is rather constant.

Pregnancy confirmation:

- Immediate increase of progesterone/pregnanetriol around the time of mating is suggestive for the right timing for breeding.
- Continuation of high progesterone/pregnanetriol level continuing for at least 16 weeks after mating is highly suggestive of pregnancy.
- Transrectal ultrasonographic examination at 8 - 20 weeks after mating allows visualization of single or twin calves. Between 10 and 20 weeks, the larger mature animal may need to lay down on its side for reliable ultrasonographic examination of the uterus. Uterine vascularization can be visualized to determine viability of the fetus, to exclude embryo absorption and mummification.
- Transcutaneous ultrasonographic examination (both flanks have a small ”window” where visualization of fetal movements may be seen) may help to determine the status of the fetus in the last months of pregnancy.
- At 6 months post-breeding pregnancy can be confirmed by elevated serum prolactin concentration. When submitting a serum sample to a lab (some human hospitals have a prolactin assay), this should always be accompanied by a serum sample of a non-pregnant elephant for comparison.

Behavioral observation at the end of gestation:

- 24 hours observation including the use of a (time-lapse) video recorder starting in week 85 may add to information about relevant events prior to parturition: night pacing, kneeling down, climbing, short periods of separation from the group, beating the vulva with the tail, frequent production of small-sized feces and small quantities of urine, loss of mucous plug, playing with mucous plug, rupture of the allantois sac, labor waves.

Predicting the time of parturition, measures and observations:

Daily blood sampling from week 89: every other a day progesterone assay, until week 91 (637 days). From week 91 (637 days): daily assays and as soon as progesterone starts to decrease: sample twice daily.

Daily monitoring of progesterone is only possible if you have a nearby facility that runs these assays on a daily basis. Find out from your nearby hospital in the early stage of

pregnancy! Many veterinary labs do not have tests that are sensitive for levels of progesterone (P4) in elephants 1-3 days prior to delivery. Also check the availability during the weekend.

NOTE: a decrease of progesterone to baseline level is not always occurring. A 23-yrs-old Asian elephant at the Rotterdam zoo (with a baseline progesterone level < 0.6 nmol/l) delivered a healthy calf while her progesterone remained above 0.65 nmol/l.

Signs of an approaching parturition may include:

- Loss of mucous plug (not seen in many facilities)
- Pre-and post-parturition ventral edema may be noticed.
- Group members may react differently (vocalizations, restlessness)
- In most cases (75%) rupture of allantois sac and loss of allantois fluid (looks like urine) is seen within 2 hours prior to birth.
- The size of fecal balls may get smaller towards the end of pregnancy.
- Frequency of urination may increase around parturition, resulting in more “watery” consistency of the urine, resembling more like allantois fluid .
- Development of mammary gland and production of milk shortly before birth is often seen, however this may also occur in a much earlier stage of pregnancy . Milk accumulation can be visualized using transcutaneous ultrasonography several hours prior to parturition.
- Softening of the pelvic ligaments (due to estrogen surge) may result in slightly abnormal locomotion of the hind legs.

Parturition/Preparations for calving:

- Training and/or desensitizing of the pregnant elephant for veterinary intervention, like blood sampling, injections, IV-infusions, milking and rectal manipulations
- If possible, store some colostrum (freezer) or store plasma obtained from the dam in weeks prior to parturition.
- Have artificial milk available (Salvana GmbH, Germany; hand raising has been done at Emmen Zoo and Berlin Zoo)
- Check restraint chains and fixation points for the legs and one extra fixation point between the hind legs for pulling devices. Soft ropes for pulling the calf away if needed should be available. The use of a calf harness has been described.
- Check the stable and place bars where a calf could possibly escape. Block all possible escaping routes for the calf (not for staff!!).
- Take out all obstacles.
- Be prepared for closing the elephant house for the public (sign post, etc.)

- Make sure there is a good stock of commercial cat litter or saw dust to be used on a concrete floor as soon as the calf is born. This will absorb much of the allantois fluids and prevent the animals from slipping on the wet floor.
- 2 or 3 pairs of keeper-gloves (soccer) to get a better grip on the wet, slippery calf when needed
- Plastic hose pipe (with pump, if necessary) for rectal cleaning with lukewarm water
- 3 birth-chains with proper handles (2 for the legs, 1 for trunk or tail); find a way to avoid back sliding when manual extraction (vaginal vestibulotomy) is required.
- Drugs to be kept in store:
  - Ca-borogluconate for I.V. infusion
  - Estradiol gel (EstroGel® 0.06%)
  - Oxytocin
  - Lidocain
  - Xylazine, detomidine or medetomidine
  - Butorphanol
  - Azaperone
  - Atipamezole
  - Doxapram
  - Oxygen
  - (Betadine®-)iodine solution for navel disinfection (umbilical infection is a major cause of perinatal complications)
  - Lubricant (many liters). J-lube®, a concentrated lubrication powder, has proven to be very useful.

The normal calving process should take place within 2 hours after rupture of membranes (release of fetal fluids).

If the plasma progesterone level decreases to below base line concentration, this is a signal that calving should take place within 48 hours. Prolonged intervals have been reported (as long as 14 days) still resulting in the birth of a living calf, but it is very likely that this is due to a disturbance of the normal birth process that should be corrected before the health of the calf is jeopardized. There is one report of a parturition taking place without a complete drop to base line level.


The normal calving process in elephants is a family happening. All efforts should be directed to making this possible. Even when veterinary intervention is required

II. Guidelines for veterinary assistance around the parturition time

Preceding any recommendation in this chapter, the following remarks need to be made:

1. It is a myth to think that a multiparous elephant does not need to be monitored and assisted during parturition according to this protocol. Too many calves have been born dead or very weak because of the fact that parturition had started unnoticed and stopped unnoticed. In our view, this protocol should be followed in ALL cases of elephant parturition as much as the elephant management allows.
2. It is a myth that oxytocin is a harmless drug to be used in elephant parturitions. In at least 3 cases the administration of oxytocin has been associated with the occurrence of an uterus rupture because of insufficient cervix dilatation. It should never be used without ultrasonographic examination of the cervix uteri. The visible presence of the allantois sac as a subcutaneous bulging mass under the tail is not a guarantee for a sufficiently dilated cervix.
3. Many elephants, including very experienced multiparous elephants may just stop the parturition process, often unnoticed. This will compromise the health of the calf. Don't relay only on what you see from the outside! For a proper judgment about the presence of labor activities or the progress of parturition, your professional judgment should be based on the results of progesterone tests and ultrasonographic examinations.
4. If you do not believe in the above mentioned statements, you may find yourself confronted with a dead elephant calf or a dead elephant mother or both. So, discuss this item with your staff and make your decision before you start breeding your elephants.
5. If the management of elephants in your zoo does not allow blood sampling or ultrasonographic examinations, you must be aware of the risks associated with a silently interrupted birth process, no matter the reproductive experience of the animal involved. Safety for the personnel should never be challenged by unacceptable risks.
6. Finally, the calving process is a natural process. Elephants should give birth in their own social

environment, excluding any external disturbing factors (e.g. noisy building in the neighborhood, visits of unknown individuals, etc.). With a well trained animal, blood sampling and ultrasonographic examinations can be done while the animal is temporarily separated from the group. Immediately afterwards, the animal should return to its group. All efforts should be made to make sure that the calf is born in the group while the mother is NOT chained! This will stimulate the acceptance of the calf by the mother and group members and is an investment for future breeding successes for the entire elephant group.

To determine the right moment when calving starts, 2 parameters are essential: the progesterone blood level and the relaxation of the cervix, monitored by ultrasonographic examination.

Progesterone: the sensitivity of the equipment and the time needed to run the assay are the bottleneck for using the progesterone concentration as a reliable tool. Today many human hospitals use advanced equipment with a very low detection level that can provide results in less than 2 hours. Make sure that you have made arrangements with a lab long before you expect the parturition.

Ultrasonography: to use this technique as a reliable tool, it is indispensable for the veterinarian to gain experience long before the elephant birth is expected. This will enable the veterinarian to distinguish a normal cervix (fig. 3a - d) from the relaxed cervix (figure 4a + b) from the normal cervix. Preferably a 3.5 MHz probe should be used transrectally.

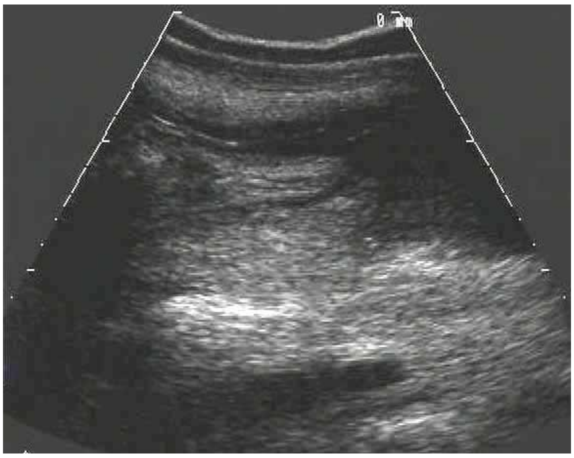


Figure 3a. Longitudinal, transrectal ultrasonographic image of the vagina and the closed cervix of a pregnant elephant.

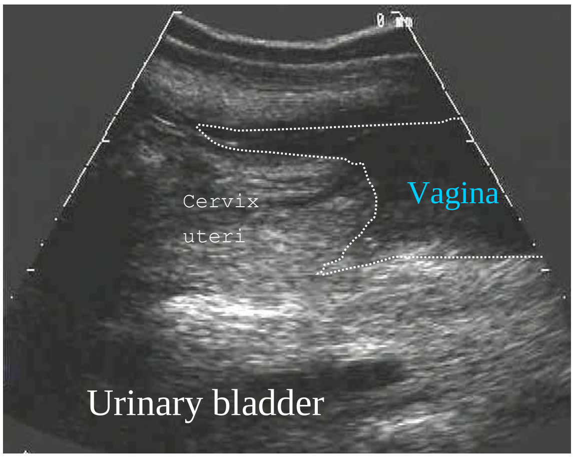


Figure 3b. The same image as figure 3a with the vagina and cervix indicated with white lines.

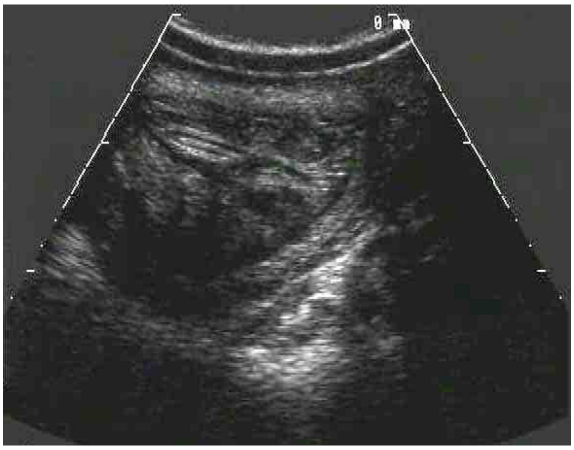


Figure 3c. Transversal, transrectal ultrasonographic image of the vagina and the closed cervix of a pregnant elephant.

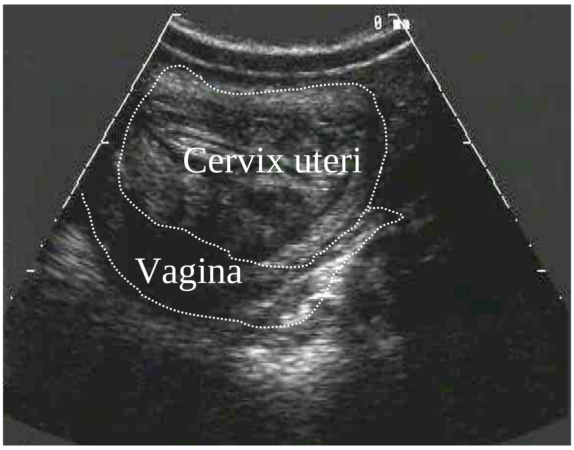


Figure 3d. The same image as figure 3c with the vagina and cervix indicated with white lines. Note the folds in the cervix uteri.

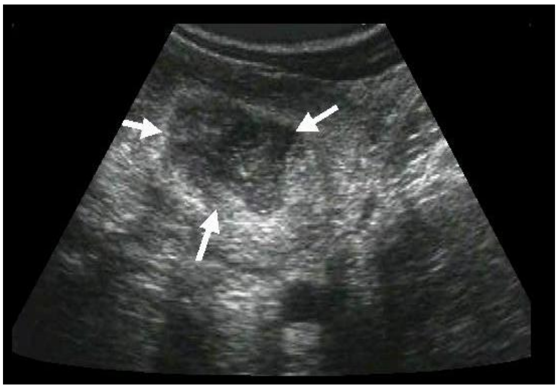


Figure 4a (transverse) and 4b (longitudinal). Allantois sac with cloudy fetal fluid in the (partly) dilated cervix of an Asian elephant 12 hours prior to delivery (white arrows: allantois sac, open arrow: pelvic bone)

During the last 2 weeks of gestation, the mucous that is present in the vagina during gestation will be discharged gradually. This is a clear indication for a pending parturition.

Recognizable onset of parturition occurs normally within 24-48 hours after progesterone has dropped to below base-line level. In these guidelines the absence of visible signs of parturition in the same time frame is considered an abnormal condition; this status requires veterinary intervention. At this point 2 situations may occur: the parturition process has started but has been interrupted without or with spontaneous rupturing of the allantois sac.

1. No rupture of allantois sac noticed:

If the calf is not born by natural way 24 hours after blood progesterone has dropped to baseline level, rectal palpation and ultrasonographic examination of the cervix is highly recommended. This will demonstrate the rate of relaxation of the cervix, the presence of the allantois sac or parts of the fetus in the cervix or vagina and should be repeated at least every 8 hours.

A blood sample should be taken to measure the calcium level. If below 2.5 Mmol/l, calcium should be administered as an IV-infusion (NB: when given in an ear vein, it should be given strictly IV in order to avoid damage to the vein) or orally (suggestion: calcium syrup concentrate for human use, enveloped in the carton core of toilet paper, covered and sealed with fresh tamarind paste has worked well; most elephants will eat it, including the carton material). The effect of the calcium administration should be confirmed by rectal palpation (increase of contractibility of the uterus) and determination of the blood calcium level. Store an EDTA and heparine sample for herpes virus diagnostic purpose (both cells and plasma in freezer after separation).

Transrectal ultrasonographic examination at 48 hours:
A. No relaxation of the cervix at 48 hours: search for calf movements and nail position of the fetus (palpation and ultrasound) and blood flow in fetal vessels (ultrasound).

Transcutaneous ultrasonographic examination (both flanks have a small ”window” where visualization of fetal movements may be seen) may help to determine the status of the fetus.

Apply estrogens rectally. Good results have been obtained by the rectal and transdermal (perineum) application of an estradiol containing estrogen gel (Estrogel 0,06%, total dose 700-800 mg estradiol). The effect on the cervix dilatation should be monitored closely by transrectal ultrasonography! At this time, at least 1 hour after the local application of estradiol, rectal massage should be applied to test and stimulate the contractibility of the uterus. Technique: remove feces from rectum, flush out the rectum, use abundant lubrification, keep both gloved hands (NB: the rectal mucosa is vulnerable due to estrogens) with the fists joined in a firm grip and press with the wrists or the dorsal sides of the hands against the pelvic ring to stimulate the pelvic receptors until strong labor waves appear or at least 10 minutes. When labor waves occur, continue this massage for 3 hours (if needed change operator). Check regularly by means of ultrasound the condition of the cervix. If there is still no cervix relaxation, continue monitoring the viability of the calf. If no fetal parts can be detected, consider the presence of pseudopregnancy (ovarian tumor, dysfunction of the pituitary gland, etc.). The application of estradiol gel (total dose: 400-500 mg 17-β-estradiol) as described above may be repeated 3-4 hours after the first treatment if the cervix dilatation is still incomplete.

B. Partial or complete relaxation of the cervix (figure 4) at 48 hours or later as a result of the situation described under 1A:

Apply rectal massage to test contractibility of the uterus. If limited or no reaction, the administration of oxytocin is contra-indicated. In this case, the administration of calcium is recommended (even when blood calcium level is within normal ranges). After 2 hours the use of estradiol as described under 1.A is recommended.

Only if uterus contraction can be provoked by the rectal massage, the use of oxytocin may be considered using the following dosage: 25-50 IU oxytocin s.c. or i.m. (if needed use a blow dart).

Oxytocin should be used with care, as it may dramatically exhaust the contractibility of the uterus muscles as well as the general condition of the female. There might also be the risk of reduced blood circulation in the umbilical chord, due to the spasms in the myometrium.

Prostaglandine E (dinoproston) has been used on a few occasions for cervix dilatation (after the administration of estradiol) and to stimulate uterus contractions. As there is still limited knowledge of its efficacy after transrectal administration and the risk of stormy uterus contractions, it should only be used when any obstructions or abnormalities of the calf can be ruled out.

Depending on the progress obtained, rectal massage and the administration of oxytocin are the 2 major treatments to follow from this point. In between these treatment events, the animal should be exercised to relieve the pain and stimulate position changes of the calf and preferably it should be kept in the group. Only when the animal cannot be separated whenever required, the cow should be kept separated from the group, but with as much physical contact as possible. Oxytocin should be given in intervals of at least 2 hours for a maximum of 12-24 hours under the guidance of ultrasound to evaluate the progress.

Continue this approach of treatment until parts of the calf have entered the pelvic cavity. If the efforts remain unrewarded and no access to fetal parts is possible, not much can be done. Continue monitoring the viability of the calf. If the calf has died, while the membranes are still intact, the risk of intoxication is limited, but immunosuppresion could be a complication for the cow. To date, no proper data are available.

The dosage of oxytocin may only be increased to 100 I.U. after parts of the calf have entered the pelvic area and progress is clearly observed. At this time, a bulge containing parts of the body under the tail of the dam should be visible. Progress of parturition must be monitored strictly at this stage. If this increased dose of oxytocin does not result in parturition a vaginal vestibulotomy should be performed soon to get better access to the calf. See next chapter.

Expulsion of the calf should follow soon after the bulge appears under the tail of the dam. The allantois sac usually ruptures during the (induced) passage through the pelvic canal.

One complication described at this stage, is reduced passage space as a result of edema in the urogenital canal resulting in a “catching effect” of the head and/or shoulder of the calf inside the soft part of the distal (vertical part) genital tract. Suffocation of the calf is a realistic complication. This condition has been observed several times in primiparous elephants of more advanced age. Elephants in this category should be prepared by the local application of J-Lube in the distal part of the vaginal vestibulum and massage of oestrogel/creme in the skin between anus and vulva. If this condition occurs, quick intervention is required by applying firm manual pressure from the outside on the calf in the sliding direction of the calf. Be aware of the risk of kicking by the mother.

2. Ruptured allantois sac:
A significant event in the parturition process is the rupturing of the allantois sac, which – when intact - acts as a hydraulic dilatator for the cervix, a natural lubrication for the dam and a pressure protection for the calf.

NB: The amniotic sac that covers the body directly, usually remains intact during the expulsion of the calf and ruptures during the final passage through the birth canal and is actively removed by the dam.

NB: a chained dam, may not be in the position to remove these membranes, possibly resulting in suffocation of the calf.

Differentiation between urine and fetal fluids is extremely difficult; smell, creatinin test strips and possibly protein concentration could be helpful.

If no progress in parturition is observed, major complications should be considered, like a dead calf, malposition of the calf (which is often dead), oversized calf, malformation and twin pregnancy. Because of the urgency of this situation, the calf should be born within 2 hours after rupture of the allantois sac and loss of allantois fluid. If not so, veterinary intervention has to take place. Two situations may occur:

<u>No fetal parts positioned in the pelvic area</u>: treatment should aim on the urgent relaxation of the cervix. Calcium status should be determined and treated accordingly (see above). The further approach is according to 1A, however the situation is more critical for both the dam and the calf.

<u>Fetal parts have entered the pelvic area</u>: Calcium status should be determined and treated accordingly (see above). Ultrasound is essential to determine which fetal parts have entered the birth canal, determine the position of the calf (visualization of the nails, posterior or anterior position, number of nails, trunk) and viability.

Malposition (e.g. only one leg in birth canal, no head while in anterior position) is an indication for vaginal vestibulotomy or fetotomy.

If no abnormalities are found during ultrasound, 50-100 I.U. of oxytocin should be given i.v. or i.m. and rectal massage should be practiced. Birth should be completed within 1 hour.

Other drugs used

Uterine laxants have been used in elephants on rare occasions. There are some anecdotal reports about the use of denaverinehydrochloride (Sensiblex®, Veyx) at a dose of 0,04 – 0,05 mg/kg BW (i.m.). Isoxsuprinelactate (Duphaspasmin. Fort Dodge Animal Health,

6290AA Vaals, The Netherlands) was used in a fetotomy case at 0,15 mg/kg BM (i.m.). Carbetocine (Depotocin®, Veyx), a long acting oxytocine has been used in 3 occasions dosage (0,09 µg/kg BW) i.m.

Post-partum care

Disinfection of the navel with Betadine® iodine is strongly recommended (if the mother allows its application).

The afterbirth usually comes off within 12 hours. There are a few reports on retained (parts of) placenta for several weeks, without major complications for the dam. Hygienic measures should be applied to reduce the infectious burden for the calf.

Be aware of the fact that a second calf can still be present in the dam. There are reports that second calves were born between several hours up to 3 months (the prevalence of twins in elephants is 1:3000).

The calf should drink (colostrum) as soon as possible, at least within 24 hours. If not, or when the calf makes a weak impression, the banked serum (or freshly taken serum) should be given to it orally. Try to find out the reason why the calf is not drinking successfully: e.g. too small, weak, painful mammary glands, malbehaviour of the dam.

If for any reason the mother is rejecting the calf, lactating herd member can take care of the calf. There is evidence that the mother takes over from this surrogate mother during the first 72 hours. If no lactating elephant is present in the herd, training for bottle feeding should start after 12 hours and continued for 72 hours. If the mother still rejects her calf, the best option for the calf is to move it to another herd with a lactating elephant. If introduction to this herd fails too, bottle feeding is the last option. To date very few bottle-raised elephants have reached the age of puberty.

III.Vaginal vestibulotomy in elephants

Vaginal vestibulotomy is a surgical procedure, in which the vertical part of the uro-genital tract (vestibulum vaginae) is exposed by a percutaneous approach.

Indications for a vaginal vestibulotomy:

- No progress in calving despite of treatment according to the guidelines for veterinary assistance around parturition.
- Malposition

Contraindication for a vaginal vestibulotomy:

- No fetal parts in the birth canal (confirmed by ultrasonographic examination and rectal palpation)

Preparations:

A. 1 extra ring in the floor between the hind legs should be present in any calving box for elephants. A pulley can be attached to it in order to provide optimal conditions for pulling the calf out (in a ventral direction) if needed. If this is no option, the calving chains (after passing through the entire vestibulum vaginae) can be attached to a long steel pipe or wooden log. Traction on the chains can be realized if 2 persons on each side of the elephant push this device downwards.

B. If not strictly needed, don't use any sedation in the cow, as you will need the cow's straining support during the extraction. If the animal is completely hands off and restraint in a chute or otherwise is not an option, use a reversible sedation (xylazine, detomidine or medetomidine/atipamezole, preferably in combination with butorphanol) to chain the elephant on 4 legs. The sedation should be antagonized (if safety permits!) as soon as straining support from the elephant is needed. Remember that the behavior may have changed during this phase of the calving most likely in favor of the veterinary intervention: the animal is probably more interested in getting the calf out than in attacking the keepers around her. But there is not sufficient information about this point. The situation has to be evaluated for each animal separately.

C. Use local anesthesia only. The incision side should be injected subcutaneously and intracutaneously with lidocain in sufficient deposits to anesthetize the entire incision site (4-5 injections of 20 ml lidocain over a distance of 20 cm) under the tail. The use of epidural anesthesia is recommended in order to reduce the movements of the tail and to support the local anesthesia. Lidocain can be injected easily with a long hypodermic needle in the intercoccygeal space. The depth of the epidural space lays approximately 6.5 cm below the skin surface. To localize the best insertion place, the tail should be moved up and down. The intervertebral space that lies in between the most cranial coccygeal vertebras that can be moved should be palpated. The needle is inserted here making an angle of approximately 60° in cranial direction. In a 3000 kg elephant, 30 ml of lidocain was sufficient to induce relaxation of the tail. The movements of the tail were minimal while the animal remained standing. A higher dose might have been tried in this case, but was not considered necessary.

D. After cutting through the skin, place a flexible plastic tube (5 cm diameter or a rumen tube) retrograde into the vestibulum vaginae to locate the incision side. To facilitate orientation, a 10x2 cm "window" should be made in the tip of the tube, 3 cm from the end. Once the skin has been incised, this window can be palpated through the subcutaneous tissue structures including the vaginal vestibulum which can be easily incised over this "window". The length of the incision should not exceed 15-20cm. This should allow the passage of your arms and the chains for pulling the calf.

E. Use normal calving chains. Don't use more than 3 people on each leg. Pulling to advance the calf in caudal direction should be done through the incision wound (a "calving pully" used in cattle has been used successfully). Once the legs of the calf have reached the wound, the chains should be advanced through the vaginal vestibulum and pulling should be realized in downwards direction allowing the passage of the calf to take place through the natural birth canal.

F. When the calf is in posterior position and can't pass through the pelvic girdle, try to rotate the calf (90° longitudinal axis) during extraction. In cattle, active rotation is the normal way of a live calf to pass its pelvis through the pelvic canal of the mother. Figure 5 (Rotterdam Zoo, 1998) shows the birth of an elephant calf after stagnation of the delivery caused by hypocalcemia, demonstrating clearly that the calf has rotated 90°. This had happened already at the time both hind legs could be palpated in the vagina. When the calf is dead, the absence of this phenomenon might be responsible for stagnation of the calving process. In the experience of one of the authors (W.S. Rotterdam Zoo, 1993) it was only possible to remove a dead calf in posterior position by vaginal vestibulotomy after 90° rotation of the calf (figure 6). Please note that at that time the calf was delivered through the surgical incision as it was not attempted to pass it through the normal birth canal.

Figure 5: Birth of an Asian elephant calf, showing the sideward rotation of the calf. (hypocalcemia, Rotterdam Zoo 1998)

Figure 6: Vaginal vestibulotomy in an Asian elephant. Extraction was only successful after 90° rotation of the dead calf. Note the umbilical chord twisted twice around the hind leg. (Rotterdam Zoo, 1993).

G. Don't hesitate to push the calf back in the direction of the uterus; it might return in a better position. During this action, both legs and the trunk or tail should be well connected to the chains. Refill the genital tract with at least 5 l. of lukewarm water ("artificial embryonic fluid") by using a pump (aquarium-type). Lots of lubricants (J-lube) should be used.

H. Don't pull at both legs in one direction at the same time. You must cross the chains and pull alternatively on each leg. It is more likely that each leg will pass the pelvic canal bit by bit, rather than that the two legs will pass in simultaneously.

I. When the calf is out give 50 IU of oxytocin i.v., flush the uterus with at least 100 l of 0.09% saline water (add 90 g kitchen salt to 100 l of tap water and use an aquarium pump with a clean hose pipe for flushing), preferably until the placenta has come off.

J. At this time (only if needed) the cow can be given a xylazine sedation (if sedated and reversed previously, use azaperone)

K. On 2 occasions a long balloon catheter was inserted into the urinary bladder and stitched to the internal wall of the vestibulum vagina. In both cases, the catheter came out within 3 days.

L. Suturing the wound is very questionable. In all known cases the wall of the vaginal vestibulum was closed in 2 layers with PDS, Vicryl or another resorbable material. In all known cases the sutures ruptured through the wound edges within 2 weeks. Repeated suturing has been attempted in most cases and has finally resulted in closure of the wound, which may take more than 1 year. In one case (after a fetotomy) a huge urinary fistula developed after 10 days located between the skin and the vestibulum vagina that drained into the outer lip of the vulva. The manipulations of the arms and chains through the wound irritate the wound edges which also does not favor natural adhesion of the sutured wound edges. Although it seems contrary to any veterinary principles, after having performed 2 vaginal vestibulotomies, it is my (WS) strong advice to initially encourage secondary healing without any suturing of the vestibulum vagina or the skin. Post-operative wound care should consist of daily flushing of the wound and the vestibulum vaginae with 50-100 l. of 0.09% saline water (900 g kitchen salt in 100 l. tap water) using a clean silicon or plastic tube fixed on a small aquarium pump. This gives minimal discomfort to the animal and will result in diminishing of the wound size by retraction of the connective tissue. In the advanced stage of natural wound healing that will certainly take place, the epidermis may adhere completely to the mucosa of the vaginal vestibulum (figure 7).

In that case splitting these 2 layers from each other under local anesthesia is required in order to enable further wound healing per secundam.

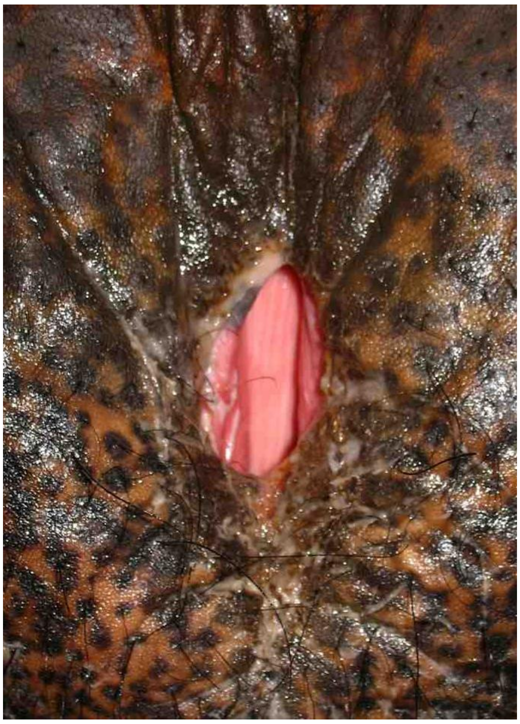


Figure 7: mucosa and epidermis are fused together, impeding further wound healing

M. Reverse the sedation, wash your hands, clean up the mess and have a beer. But **DON'T CLOSE THE SKIN.** Forget everything you learned about surgery. Whatever fancy suture technique you may use, the skin wound will open again. Don't think that your special suture will work, because every possible suture technique has already failed. Even when closing the skin, feces will still contaminate the wound, because the skin property does not allow a watertight wound closure; suturing the skin has proven to result in discomfort for the elephant, the need to repeat the procedure several times and finally a permanent fistula just as large as in case the skin was not sutured. The skin will heal per secundam; periodic refreshing of the wound edges may be required during the healing process.

N. Give antibiotics during at least 7 days.

O. If you were not able to remove the calf: **DON'T TRY TO PERFORM A CESAREAN UNLESS YOU ARE ABSOLUTELY SURE THAT THE COW WILL NOT SURVIVE CONSERVATIVE TREATMENT**. All cesareans performed till now have resulted in the death of the mother. If you see yourself facing the critical situation that the calf can not be removed despite of the vestibulotomy and fetotomy is either no option or it has failed, the first advice is to leave the wound of the vestibulotomy open completely and see what happens during the days to come. Flush the uterus frequently with large amounts of 0.09% saline water water with a disinfectant like Betadine® iodine to prevent the occurrence of Bandl's rings that may cause necrosis of the uterus. An antibiotic treatment is recommended.

Fetotomy

Only very few cases have required fetotomy. If you decide that a fetotomy is needed, you are strongly advised to contact Thomas Hildebrandt and/or Willem Schaftenaar.

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APPENDIX III

Emergency care for elephants clinically ill from Elephant Endotheliotropic Herpes Virus–hemorrhagic disease (EEHV-HD)

Fieke Molenaar (ZSL-Whipsnade zoo), Mads Bertelsen and Kathryn Perrin (Copenhagen zoo), Imke Lueders (GEOLifes), Lauren Howard (Houston zoo), Willem Schaftenaar (vet adv. Eur. Elephant TAG)

17 August 2020

(Based on the treatment protocol of Houston Zoo EEHV and the Asian EEHV Working Group)

Time is essential when treating elephants with EEHV. Extremely sick calves and juveniles may not look particularly ill, and may eat, drink, and participate in training, until literally moments before they die. Waiting until the animal looks very sick is associated with a poor prognosis and death. Even if a young elephant looks only mildly ill or uncomfortable, veterinarians and caretakers are strongly urged to **start rectal administration of fluids**. This technique can be life-saving because what appears to kill young elephants suffering of EEHV-HD is vascular shock. Rectal fluids can alleviate the early physiological effects of shock and prevent the spiralling of events that leads to death.

1. Collect baseline information

- Anamnesis: acitivity pattern, appetite, sleeping pattern.
- Physical examination (body posture, evidence of oedema around eyes, head, neck and ventral abdomen, temperature, blood pressure, **changes in color or ulceration of mucous membranes,** buccal mucosal bleeding time, etc);
- Blood collection: whole blood (EDTA, heparin and citrate), serum, and blood smear for PCR/qPCR, hematology, WBC (including platelets count!) and chemistry; samples should also be stored for future research (please store any leftover blood collected).
- Contact the nearest diagnostic lab that runs PCR and qPCR on all relevant genotypes for emergency diagnosis and arrange sample transport (see address below).
- Blood samples should be tested DAILY using qPCR in order to adjust the treatment regime according to the viral load.

European labs that can perform EEHV qPCR
Date: 17-08-2020

Universität Berlin, Institut für Virologie
Robert von Ostertag-Str. 7-13, 14163 Berlin, Germany
Prof. Dr. Klaus Osterrieder/ Dr. Jakob Trimpert/ Dr. Azza Abdelgawad
qPCR for: EEHV-1, 2, 3/4, 5 and 6 (Asian and African elephants)
Tel: (+49) 30 838 59780 / +49 30 838 67281
Email: **no.34@fu-berlin.de** / **trimpert.jakob@fu-berlin.de** / **azza.abdelgawad@fu-berlin.de**

Rotterdam Zoo, Veterinary Department.
Postbus 532, 3000AM Rotterdam, the Netherlands
Linda Bruins-van Sonsbeek DVM; Christine Kruger-Velema
qPCR for: EEHV-1, 2, 3/4, 5 and 6 (Asian and African elephants)
Tel: (+31) 10 4431485 (+31) 10 4431541
Email: **l.van.sonsbeek@diergaardeblijdorp.nl** / **c.kruger-velema@diergaardeblijdorp.nl**

SCG Diagnosztika Kft / UVM Large Animal Diagnostic Centre
H-2225 Üllő, Dóra major, Hungary
Biksi Imre DVM ; Albert Ervin PhD; Dán Ádám PhD
qPCR for: EEHV-1,2,3/4 and 5 (Asian and African elephants)
Tel: (+36) 309487747
Email: **scgdiagnosztika@gmail.com**

Irish Equine Centre
Johnstown, Naas, County Kildare, Ireland, W91RH93
Ann Cullinane MVB PhD MRCVS
qPCR for: EEHV1, EEHV3/4 and EEHV5 (Asian elephants only)
Tel: (+353) 45 866266
Email: **acullinane@irishequinecentre.ie**

Chester Zoo, veterinary Department
Chester CH2 1EU, United Kingdom
Javier Lopez DVM
qPCR for: EEHV1 and 3/4 (Asian elephants only)
Tel: (+44) 1244 389757/(+44) 7880 242006
Email: **j.lopez@chesterzoo.org**

2. Essential sample collection and first treatment may need standing sedation:

- Standing sedation can be performed using detomidine in combination with butorphanol.
  - Detomidine 0.01-0.022 mg/kg IM (can be reversed by atipamezole at 3 times the dose of detomidine)
    AND
  - Butorphanol 0.045-0.075 mg/kg given at same time as detomidine. Butorphanol can be reversed with naltrexone at 2.5-5 times the dose of butorphanol in emergency situations, but reversal is not essential and should preferably not be carried out if the calf is considered to be in pain.

  OR

  - Xylazine: 0.04-0.08mg/kg IM (can be reversed with yohimbine or atipamezole)

- Provide supplemental oxygen via nasal cannula whenever possible.

3. Essential supportive fluid therapy:

- Rectal administration of lukewarm, clean water is the first choice of fluid therapy in sick calves. It should be given through a garden hose or rubber tubing after careful removal of fecal balls from the distal part of the rectum (use sufficient lubricant in order to avoid irritation of the rectum mucosa which causes peristaltic activity). When the hose is placed over the horizontal ridge in the rectum (approximately 1 elbow length from the anus), the tube can be advanced for another 100 cm (if possible). A gastric pump can be used; if not available use a large funnel.

  Rectal fluids should be administered a minimum of 3-4 times per day, up to every 2 hours. A bolus treatment of 10 to 20 ml/kg dose is often used. When finished, the tail should be held down for at least one minute.

- Placement of an intravenous catheter (16-20G IV catheter, with a minimum length of 6 cm to prevent perivascular leaking) in a large, peripheral vein is recommended for:

  - Plasma transfusion (supplementation of platelets!) after cross matching recipient blood with donor plasma at 0.5-2 ml/kg BW (see fact box below). The donor should be an adult elephant, preferably PCR-screened on EEHV-viraemia at the time of blood collection.
  - Administration of other iv-only medications. Please note that the ear veins are very susceptible to vasculitis, associated with perivascular administration of drugs. Sloughing off of the ear pinna distal to the affected vein is likely in these cases. Extra care should be taken with drugs that are particularly caustic.
  - Iv fluid therapy, which will require follow up with rectal fluids.

4. Antiviral drug administration Antiviral drugs are thought to have an effect during the early stages of viral replication. It is therefore recommended that antiviral theraphy starts as early in the process as is feasible. However treatment may also be attempted in acute cases. The efficacy of the following drugs has not been proven, but all survivor cases have been treated with one or the other of the following drugs:

- **Famciclovir:** 15 mg/kg orally or rectally TID (grind with mortar and pestle, mix with water to make into a paste and further dilute with water).

    - Medications should not be administered rectally within one hour of rectal fluid administration.

- **Ganciclovir**: in advanced stages of the disease, when a reduced absorption from the intestinal tract can be expected, intravenous administration can be considered more prudent and slow i/v administration of gancivlovir at a dose of 5 mg/kg BID (dissolved in 1 liter of fluid during 1 hour) should be considered.

- **Acyclovir:** therapeutic doses have yet to be established, but 15mg/kg BID was used in a survivor case: orally, rectally (grind with mortar and pestle, mix with water to make into a paste and further dilute with water) or intravenously.

5. Antibiotic administration

Antibiosis should be considered for treatment of underlying conditions or secondary infections associated with leucopenia and immunosuppression:

- Ceftiofur: 1.1mg/kg IV BID
- Enrofloxacin: 2.5mg/kg PO or rectally SID
- Marbofloxacin: 2mg/kg IV, IM, SQ SID has been used
- Amoxicillin: 11mg/kg IM SID
- Penicillin G: 20,000-50,000 IU/kg IM or IV TID-BID (BID administration has been used in EEHV survivor cases in Asia)
- Any suitable antibiotic with presumed action against invasive gut flora

6. Plasma transfusion

Plasma is currently considered one of the best supportive therapies to provide, as platelets, clotting factors and potentially protective antibodies can thus be provided. Note that the freezing process activates platelets, which renders them useless at the time of transfusion. Therefore - where possible - freshly collected plasma is preferred. The following should be considered for plasma transfusions:

- If frozen plasma is available, this can be given in an early stage of the disease to save time (despite the activated and spent platelets).
- Blood collection from an adult elephant (plasma donor) should be initiated to provide fresh plasma as soon as possible.
- Cross-matching the donor animals with the recipients, especially if one donor will be used on multiple occasions (see text box below).

Cross-match

Based on design elaborated by Houston Zoo, Inc.

Step one: Prepare a 3-5% red cell suspension.

1. Collect blood from both donor and recipient in EDTA.

2. Centrifuge the tube and separate the plasma from the red cells. Save both.

3. Place 1 drop of recipient red cells into a small (2-5 ml) clean test tube.

4. Add approx. 1-2 ml of normal saline to the tube with the red cells (or 1 drop RBC to 40 drops saline)

5. Centrifuge at 2500 RPM for 20 seconds.

6. Remove the supernatant, leaving the red cell button on the bottom.

7. Repeat steps 4-6 three times (for a total of 4 washes).

8. Add 1 drop of newly washed recipient red cells to a new test tube.

9. Add approximately 20-40 drops of saline and mix to suspend the red cells. This should be an approximate 3-5% cell suspension to work with.

Step two: Minor cross-match (for plasma transfusion).

1. Add 1 drop of the recipient's 3-5% red cell suspension to a labeled test tube. Add 1 drop of the recipient's 3-5% red cell suspension to another labeled test tube to be used as a control.

2. Add 2 drops of donor plasma or serum to the test tube.

3. Add 2 drops of saline to the control tube.

4. Incubate these tubes at 37^{o}C for 15 minutes.

5. Centrifuge the tubes for 20 seconds at 2500 RPM.

6. Observe the supernatant for signs of haemolysis. If present in the cross-match tube and not the control tube, the match is not compatible. If present in both, start again with a new cell suspension.

7. If no haemolysis, then gently rock the test tube back and forth to re-suspend the cell button. Observe the cell button

- PCR screening the donor plasma for current EEHV DNA;
  Best practice requires screening of donated plasma for EEHV-DNA by qPCR at the time of collection, prior to administration. Depending on logistical challenges and laboratory availability, there is the possibility that this may delay plasma donation by several hours to two days, which may be detrimental in urgent (severe clinical) cases. If a donor has been screened routinely on a regular basis prior to donation of plasma, this will greatly reduce the risk of introducing more (or different) virus to the calf. It is therefore prudent for collections with at-risk juveniles to identify possible donor elephants (from neighbouring collections if required) and collaborate in advance.
- *If stored, storage at -80°C is essential;*
- Plasma separation does not require a centrifuge. Leaving to stand overnight followed by manual separation (see below) is feasible.
- For administration of plasma, a patent iv cannula and a filtrate infusion giving set are required.
- Dose rate 0.5-2 ml/kg/day – the first 100 ml of each donor should be given slowly to monitor for anaphylaxis.

7. Administration of recombinant Factor VII may be considered if the elephant has reached the state of hypo-coagulation (blood is not clotting anymore) post-DIC. NovoSeven© has been used in a case at Copenhagen zoo. The drug appeared to improve coagulation, but the calf still died. The drug is prohibitively expensive, but worth further exploration. Suggested dosage: 0.07 mg/kg IV TID, but effect should be monitored using Thrombo Elastography (TEG) or similar.

8. <u>Pain relief</u>

Opioids are a useful adjunct to providing pain relief and in some cases mild sedation to assist in the management of animals being treated. There is the possibility of behavioral changes in the elephant when using opioids and trained behaviors may well be lost or less responsive. A dose of 0.008-0.014 mg/kg butorphanol IM (repeat every 3-4h) is recommended for analgesia.

<u>9. Adjunctive therapies to consider</u>

A single high dose of a short-acting glucocorticosteroid can be considered, as the vascular damage might be caused by an overenthusiastic immune response of the host:

- **Flumethasone: 0.005 mg/kg IV or deep IM**
- **Dexamethasone 0.05-0.5 mg/kg IV or IM**
- **Triamcinolone 0.067 mg/kg IV (dosage given in 1 survivor in Thailand,** *Note:* **This data is under discussion)**

<u>10. Anti-inflammatory Drugs:</u>

- Although EEHV is thought to be a vasculopathy as opposed to a vasculitis, anti-inflammatories are indicated as part of the analgesic regime as well as reducing secondary inflammation resulting from peripheral edema and hemorrhage. Non-steroidal anti-inflammatories (NSAIDs) may play a useful role in early management of the disease. However it should be noted that in human medicine NSAIDs are contraindicated in cases where peripheral edema or hemorrhagic diathesis is present due to the decreased glomerular filtration rate and the effects on coagulation seen when using NSAIDs. The analgesic and anti-inflammatory effects of these drugs should be weighed against these possible side effects. Flunixin meglumine or other NSAIDS should be administered to patients that appear hydrated or are receiving rectal or IV fluids. Administration of omeprazole for gastrointestinal protection during NSAID treatment should be considered. The equine dose is 0.7 - 1.4 mg/kg PO once daily.
    - Flunixin meglumine 0.25 to 0.5 mg/kg IV/IM SID
    - Meloxicam 0.2mg/kg IM SID has been used
    - Ibuprofen 6mg/kg PO BID
    - Phenylbutazone 3mg/kg q48 hours (published dose), 1-2.5mg/kg SID (anecdotal dose) or suxibuzone (loading dose 6 mg/kg/day followed by 3 mg/kg/day)

INTENSIVE CARE OF THE EEHV-HD PATIENT

In any suspected or confirmed EEHV-HD case aggressive supportive therapy and close monitoring of the patient is essential. Rectal administration of fluids (water) is the treatment of first choice. Placement of an intravenous catheter in a large, peripheral vein is recommended for plasma transfusion (supplementation of platelets!) after cross matching recipient blood with donor plasma and administration of other medications. **The access to veins should not be jeopardized by unnecessary administration of drugs that can also be administered via another route.** If treatment is not possible under training or manual restraint, sedation will be required.

Antiviral medication is recommended to reduce or eliminate viral replication and thus reduce the viral load in the patient. Although there is no hard evidence that the antivirals mentioned in this protocol are effective, they are recommended until proven that they don't work.

Sedatives may be administered to facilitate treatment and to manage pain. Low doses of butorphanol have been safely used in clinical cases. Antibiotics have no effect on viral infections, but must be given to affected animals to prevent and treat underlying and/or secondary infections. If possible, the initial dose should be administered intravenously. Following cessation of intravenous treatment, a change to intramuscular or oral products will be made if appropriate.

Light Sedation in Adult Elephant

- It may be necessary to sedate the dam or other adult herd mates so they are not stressed during manipulations of a calf
- Butorphanol 0.006 mg/kg IM and detomidine 0.0026 mg/kg IM
- Sedation can be reversed as described above but is not necessary
- Alternatively, xylazine or other sedative agents can be used if detomidine is unavailable.

Intravenous Catheter Placement

A temporary IV catheter (16-20 G, minimum 6 cm length) may be placed in the ear, rear leg, or front leg.

Please note that elephants in an intensive care environment can be subject to secondary infections. Attention to hygiene and biosecurity is very important in elephants being treated for EEHV HD, particularly due to their immuno-compromised status.

Intravenous Fluid Therapy

A bolus of IV fluids (0.3 to 1 ml/kg in a calf) can be given to a dehydrated or "shocky" elephant as a resuscitative measure; this bolus could be repeated up to three times with re-evaluation of the patient and vital signs after each bolus. Asian Elephants have very low serum osmolarity and are hyponatraemic and hypochloraemic compared to other species. IV fluids should always be followed with large amounts of rectal fluids (tap water).

Plasma Transfusion

Colloids, such as fresh or frozen plasma or hetastarch are often more effective than crystalloid fluids for volume expansion in viraemic or seriously ill animals. The larger molecules in these fluids do not leak out of capillaries as easily, and increase plasma volume. In this respect, a (fresh!) plasma transfusion has high priority as it provides thrombocytes and coagulation factors. As the preparation of fresh plasma is time consuming, banked plasma can be

administered as emergency treatment. To supplement platelets, frozen plasma is NOT suitable, because it contains *activated* thrombocytes, which will be useless in case of Disseminated Intravascular Coagulopathy like EEHV-HD. The best plasma to administer is so called Platelet Rich Plasma (see below). In addition, plasma from a donor with a high antibody titer may help bind up virus particles in the patient although the role of antibodies is not yet well understood in EEHV-HD. Plasma should only be administered intravenously after (minor) cross-matching donor plasma and recipient whole blood samples to assure compatibility. Additionally, it would be ideal if the donor animal's blood be PCR tested to ensure the donor does not have a high EEHV viraemia. This information would also be useful as retrospective information. As there will probably be no time for PCR-screening, this can be done later on a stored sample. (Frozen plasma should be checked for the presence of EEHV (PCR) prior to collection or freezing.) The first 100 ml should be given slowly, and heart rate, respiratory rate, and temperature should be monitored. Possible transfusion reactions include fever, rash, or anaphylaxis. Mild signs can be treated by decreasing the rate of transfusion. More severe reactions should be addressed by stopping the transfusion. If no reaction is seen, the transfusion rate can be increased to 0.5-2 ml/kg BW. Clinical improvement may be seen at a plasma dose of 0.5 ml/kg.
Resuming: use banked (frozen) plasma for emergency treatment (coagulation factors, antibodies, colloids) and start preparing for fresh plasma (platelets, coagulation factors, colloids).
Please note that a major cross match needs to be carried out if whole blood is transfused, instead of just plasma.

How to collect platelet rich plasma without specific blood bags:

A. Collect blood in a container with acid citrate dextrose (ACD) as an anticoagulant at the ratio of 6 to 1 and mix gently. In the absence of specific blood bags, empty NaCl-infusion bags or plastic infusion bottles can be used (maintain sterility!) The sample can be kept at room temperature (20-25°C).
B. Instead of ACD, heparin can be added to the donor blood (6,250 IU heparin/liter whole blood)
    1. Centrifuge at 200G for 10 minutes at room temperature
    2. Remove plasma and change to a new tube.
    3. Centrifuge at 1,650G for 10 minutes
    4. Platelet rich plasma (at bottom of tube) can be kept at 4°C and be used within 5 days.
    5. If heparin was used as anticoagulant, this can be reversed by protamine HCl (10 mg protamine HCl/1,000 IU heparin given IV).

<u>Oxygen Therapy</u>

Supplemental oxygen therapy should be administered, when possible, to all patients with clinical signs undergoing treatment for EEHV. Oxygen can be administered at 2-4 l/minute via a flexible tube passed into one side of the trunk. If the elephant will not tolerate oxygen therapy while awake, it may be possible to slip the tube into the trunk while the elephant is sleeping.

EQUIPMENT AND SUPPLIES

The following equipment and supplies will need to be on hand for support during therapy.

Drugs and Equipment needed:

- ✓ Banked plasma (frozen at -80°C)
- ✓ Antiviral (Famciclovir, ganciclovir)
- ✓ Sedatives (Detomidine, Butorphanol, xylazine)
- ✓ Reversals (Atipamezole, Naltrexone)
- ✓ Antibiotics (Ceftiofur, Penicillin, Amoxicillin, Enrofloxacin, Cephalexin, etc)
- ✓ Mineralo-corticosteroids
- ✓ NSAIDs (Flunixin meglumine, Meloxicam, Ibuprofen, phenylbutazone, etc)
- ✓ Plasma transfusion set
- ✓ “Plasma extractor” (see below)
- ✓ IV fluids
- ✓ Syringes
- ✓ Needles
- ✓ 16-20 GA catheters, min 6 cm length
- ✓ Rectal fluid Kit (tube and gastric pump or large funnel)
- ✓ IV administration sets with injection ports
- ✓ Standard Extension set
- ✓ Tape for holding catheter in place + skin glue
- ✓ Stethoscope
- ✓ Thermometer
- ✓ Mortar and Pestle
- ✓ Exam gloves
- ✓ OB sleeves and lube
- ✓ Gauze
- ✓ Flashlights/ head lamps
- ✓ Towels
- ✓ Inner tubes (various sizes)/ gym mats —to be used for cushioning and support in the event of a full immobilization procedure
- ✓ Surgical prep: Chlorhexidine scrub or Povidone iodine and alcohol
- ✓ Oxygen bottles and regulator

Placement of an intravenous cannula

into an ear vein in a juvenile Asian elephant

4a 4b 4c 4d

Placement of an ear vein cannula in a juvenile Asian elephant
a) aseptic preparation of the ear pinna after numbing the area with EMLA cream one hour previous, b) insertion of the cannula after cutdown of the skin with a scalpel blade, c,d) fixing the cannula to the skin with skin glue, e) attaching the giving set and creating a loop to prevent removal of cannula on movement of the head, f) fixing the giving set to the head, g) boluses of medication can be given swiftly through giving set ports, e.g. fluids and antibiotics, h) antivirals, fluids and nutraceuticals can be given slowly

4e 4f 4g 4h

ZSL | WHIPSNADE ZOO

In-House Plasma Separation Procedure for elephants

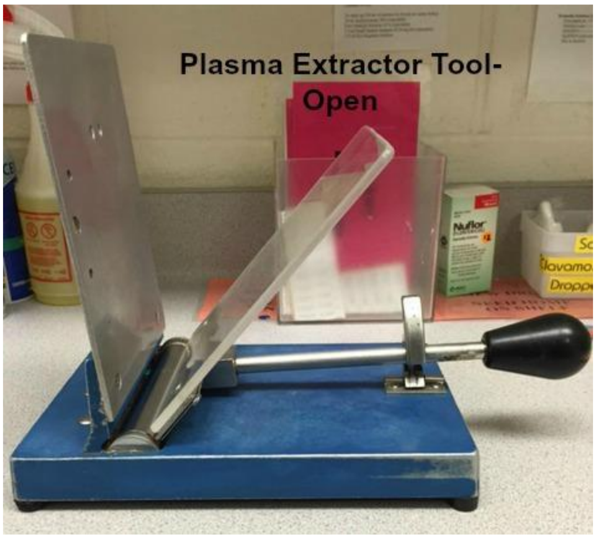
Design elaborated by Houston Zoo, Inc.

How to make a "Plasma Extractor"

If you do not have one of these manufactured Plasma Extractors

You can make one!

Material:



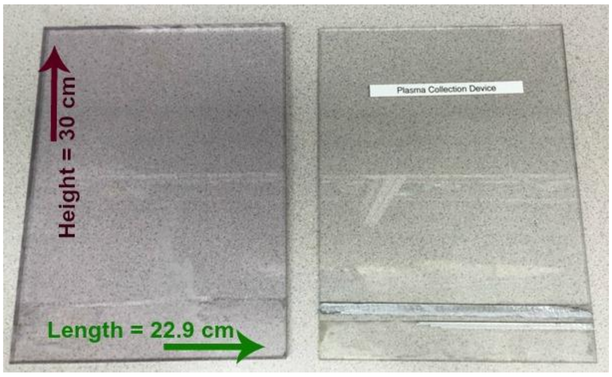
- ✓ Two pieces of Plexiglas
- ✓ Duct Tape

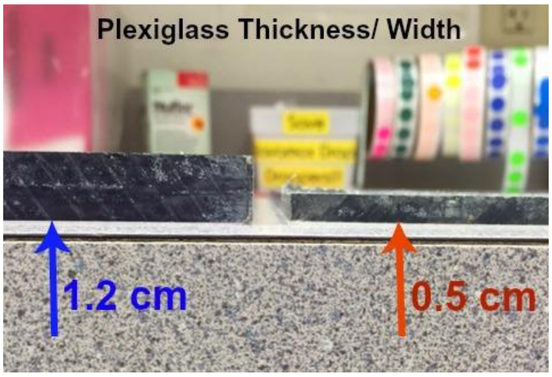
Procedure

Step 1: Prepare two pieces of Plexiglas to match the following measurements (note difference in thickness to provide sturdiness).

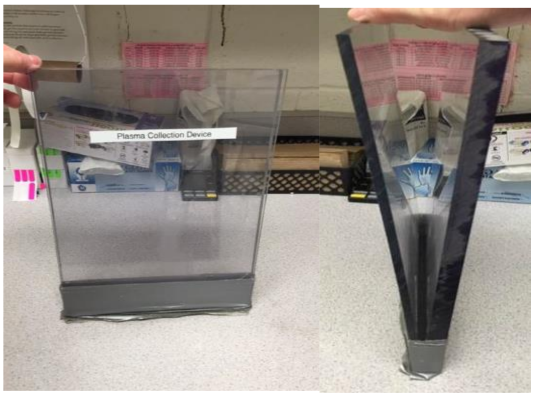
1st piece: Length= 22.9 cm, Height= 30 cm, Width= 1.2 cm

2nd piece: Length= 22.9 cm, Height= 30 cm, Width= 0.5 cm





Step 2: Align the pieces of Plexiglas together evenly and hold them together. Then wrap duct tape around the bottom ends of the pieces to keep the Plexiglas together.



*Make sure that you can pry the untapped edges apart. The Plexiglas must be able to part wide enough for a full bag of whole blood to fit in between the pieces.

In-House Plasma Separation Procedure for elephants.

Based on design elaborated by Houston Zoo, Inc.

Materials

- Sterile blood collection bag containing anticoagulant Citrate phosphate dextrose adenine solution (CPDA-1) USP for collection of 450 ml of whole blood. *Establish weight of the empty plasma bag prior to collection.
- Refrigerator with temperature 0-4 °C
- Scale (g)
- Plasma Extractor
- 1-2 Kelly or Crile Hemostats
- 1 smooth-jaw Hemostat
- Plasma Extractor – Handmade vs. advertised
- Hand-held Blood Bag Tube Stripper/Cutter/Sealer tool
- 4 Plastic Clamps
- Metal Clips **Establish weight of a single clip.

Procedure

1. Receive bag of whole blood with Citrate phosphate dextrose adenine solution (CPDA-1) USP coagulant.
2. Hang the bag in refrigerator for 6-24 hours to allow for gravitational separation of plasma from red cells. Temperature should be between 0-4 °C. (Figure A)
3. Carefully remove the blood bag from the refrigerator. Avoid re-suspending the separated red blood cells into the plasma (minimize abrupt motions when handling the collection bag).
4. Begin plasma separation process by inserting the blood bag into

    a.) the “Plasma Extractor” or

    b.) 2 pieces of Plexiglas duct-taped together. Lay the empty plasma bag beside the extraction apparatus. (Figure B)

5. Break the plastic barrier piece connecting the blood bag to the empty plasma bag. (Figure C)

6. With one hand, slowly apply gradual pressure to the Plexiglas pieces and with the other hand, use hemostats to hold the connection tubing. The plasma from the blood bag should be flowing into the plasma bag. Be cautious of disrupting the sediment. (Figure D)

7. When most of the plasma has separated into the plasma bag, quickly clamp off the connecting line with hemostats. Add secondary plastic clamps for extra security. (Figure E)

8. Using the handheld stripping tool, begin easing the remaining plasma in to the collection bag. (Figure F)

9. Using another set of hemostats, clamp the line closer to the plasma bag, leaving approximately 30 cm of tubing. Add secondary plastic clamps if necessary. (Figure G)

10. Cut the connecting line so that the plasma bag separates from the blood bag.

11. To properly seal the plasma bag for storage, tie 1-3 knots at the open end of the tubing. (Figure H)

12. Make a loop with the tubing and apply 2-3 evenly spaced metal clips. (Figure I) Slide the first metal clip as close to the bag as possible. Clamp the clips down with the multi-tool. (Figure J)

13. Weigh the full plasma bag. To determine actual plasma volume, subtract established materials weights (empty plasma bag* and # metal clips**) from the weight of the full plasma bag.

14. Label the plasma bag with animal ID number, collection date and plasma volume.

15. Store the plasma in a freezer (preferably -80 °C). However, use fresh plasma for treating EEHV-HD as freezing will activate the thrombocytes, making them useless for EEHV-HD treatment.

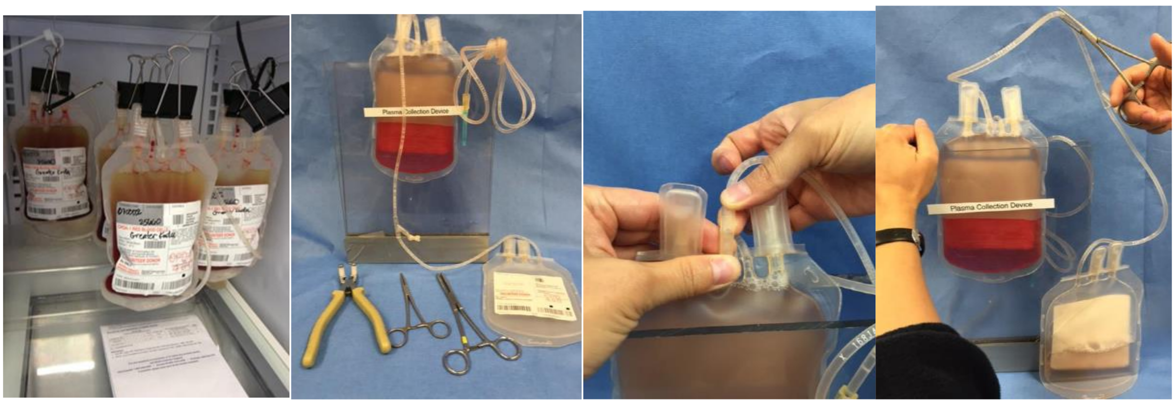


Figure A-D

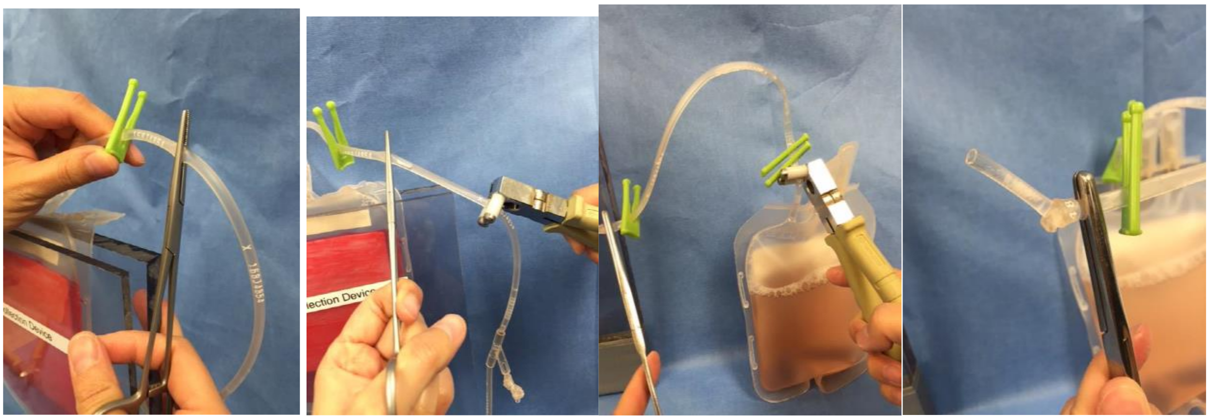


Figure E-H

Figure I *Figure J*

APPENDIX IV

Demonstration Guidelines for elephants used in public demonstrations

The ‘Guidelines on the use of animals in public demonstrations’ (September 2014) provides guidance on the use of exotic animals in public demonstrations at EAZA member institutions. The EAZA Elephant TAG supports these has, thus far, used this as the model in the absence of taxa specific guidelines. To best support our members on best practice guidance, the EAZA Elephant TAG has in addition to the EAZA Best Practice Guidelines for Elephants produced this ‘Demonstration guidelines for elephant species used in demonstrations’ document to provide species-specific guidance for institutions performing public demonstrations with elephants.

Public demonstrations are defined as any case where an animal is demonstrating behaviours, trained or natural, while under the supervision or control of a trainer in the view of guests, with the intention of educating, inspiring, and entertaining the visitors (EAZA, 2014). A public demonstration should be designed with animal welfare as the primary factor and the visitor experience as a secondary factor and should utilize the best practice standards. Following the definition of Good Practice in the ‘EAZA position statement on circus membership of the association’ (April 2017) elephant demonstration programmes should focus on behaviours that are demonstrations of an animals’ natural intellectual or problem-solving abilities and their physical attributes, showcasing as much behavioural diversity as possible. All public demonstration programmes with elephants should reflect section 1.11.2 of the ‘EAZA standards for the accommodation and care of animals in zoos and aquaria’ document (2014) and serve to:

- Promote an understanding and raise awareness of elephant species (including medical training and demonstrating anatomic specialities like the horizontal progression of the molars, species differences in the fingers of the trunk, toe nails, tusks etc.).
- Allow the visitors to see the elephants active and highlight elephant species natural behaviours for example holding volume of trunk, spreading ears to show vessel for blood cooling, demonstrate feet to explain feeling of seismographic waves as means of communication etc.
- Educate visitors about their special adaptations and their role as ecosystem engineers.
- Educate the visitors about the biology and social behaviour of elephants, the threats that most of elephants in the wild face, the role of zoos in the conservation efforts of elephants, the role of zoos generally in the global effort to preserve animals and their habitats in the wild, as well as the efforts and challenges to manage these animals in captivity.
- Inspire guests to connect with elephant species with the hope of conservation minded behaviour change for eg. mention the ivory problem as main threat of elephants in Africa (every 15 minutes an elephant is killed for ivory)!

The Elephant TAG does not support the performance of any behaviours for the purpose of a demonstration that when implemented poses a demonstrable or probable risk towards animal and / or human health or welfare. These include:

- Any situation that is demeaning or degrading to the animal.
- Any practice that requires the physical disciplining of an animal to provide protection for a staff member who is in contact with that animal for any purpose other than the preservation or improvement of its health and wellbeing.
- Any situation that requires members of the herd to be separated for the sole purpose of the demonstration.

The Elephant TAG recognizes any kind of behaviours which could be utilized for medical training (e.g., body parts presentation, touching targets, holding for examination and blood draw training) as part of husbandry training. The behaviours can be performed as part of a public demonstration to serve to facilitate the inspection of the animal and (in special cases) also to the drawing of samples or the treatment of a trained individual. Actual medical procedures should not be part of demonstration, but the Elephant TAG recognizes that desensitization (e.g. fake needle touches, the use of ultrasound probes) could be part of an educational talk about veterinary care and protected contact. For information on species-specific behaviours and recommended husbandry/safety procedures check the relevant EAZA Best Practice Guidelines or contact the EEP Coordinator for further advice.

The following points are strongly recommended by the Elephant TAG to be followed and should be taken into account prior to setting up a public demonstration with elephant species:

Training of animals

1) The Elephant TAG is strictly against any use of rearing techniques for demonstrations that directly affect the welfare and health of the animal, including the premature removal of an animal from the mother with the intention of hand-raising specifically for use in a demonstration. This could lead to unacceptable imprinting on humans and a welfare issue in them not being entirely socialized to conspecifics (Health of animal).

2) Training techniques used for demonstrations must be based on positive reinforcement to promote animal welfare.

3) There should be an emphasis on the use of motivating operations whereby the animal is seeking out food in a natural way, not reliant on the hunger state. (This is referred to in the use of “weight control” in the EAZA animals in demonstrations guidelines).

4) The elephants should always be able to choose if they want to participate in the demonstration. There should be no repercussions in food, choice or enrichment if they choose not to participate.

5) Any behaviours that are not natural or part of a medical or health prophylaxis training should not be trained for including animals sitting down or standing on their front legs and similar types of circus-style behaviours.

Safety and housing

The Elephant TAG does not support placing elephants in a performance environment that does not reflect the EAZA Standards for the Accommodation of Animals in Zoos and Aquaria (2014).

6) The elephants should not be moved outside their enclosure for the purpose of demonstrations because of animal welfare and safety reasons. Situations where animals need to be moved temporarily or permanently from their current enclosure to another enclosure / location (i.e.

for veterinary purposes, crate training, socializing / reintegrating in the herd) should not be done as part of any public demonstration to ensure the upmost animal welfare.

7) Given that elephants are classified as hazardous animals (EAZA Standards, 2014), free contact management should be excluded for all demonstration's purposes in elephants due to safety considerations. In alignment with this staff, mahouts or guest must not ride on elephants.

8) Members of the public should not be taken into the same enclosures with elephants present as it can greatly compromise human safety and animal security.

9) The physical touching of elephants by the public is not accepted by the Elephant TAG (Human/Animal interaction). Situations where the demonstrator has to touch an animal as part of a veterinary/ medical training procedure i.e. touch the feet to condition for foot-care, that is visible by the public would be accepted however the purpose of such an activity should be made clear to the public via signage or personal communication. It should be made clear that the touching of animals by public/guests in the company of an animal keeper could encourage other members of the public that are viewing this activity to attempt to touch elephants through fences when no keeper is present and thus compromises public safety.

Usage of props

10) Walking elephants is not acceptable by the Elephant TAG (Human/Animal interaction). It sends the wrong message to the public about these wild animal species being tame or even domesticated. This action also has the potential to increase risk of human/ animal injury should the animal break training and/or run away from the demonstrator.

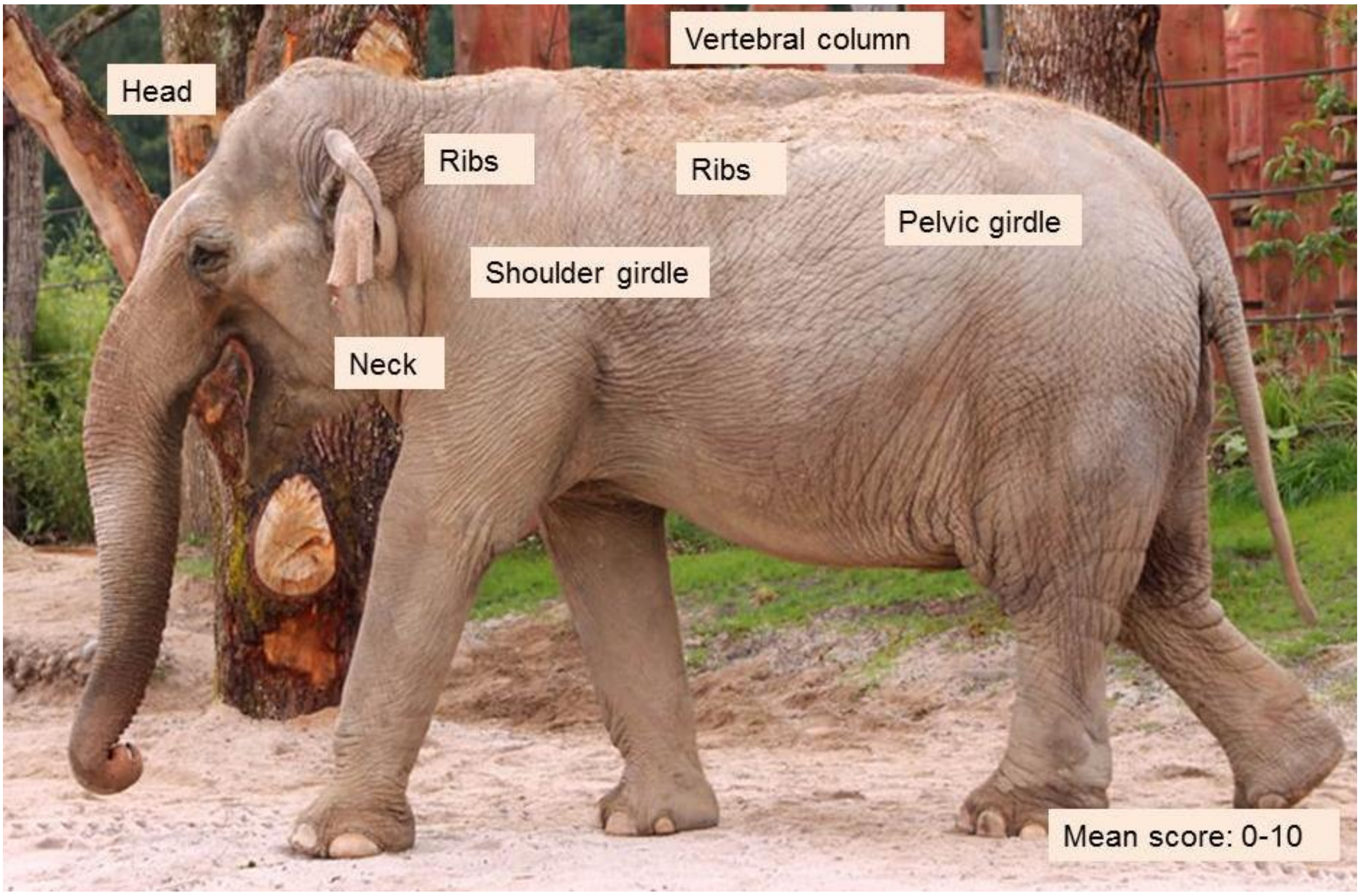
11) The Elephant TAG does not support the use of props (any item/ object used by the animal or trainer as part of animal demonstration or training programmes) that compromises animal welfare.

EAZA Elephant TAG (February2020).

APPENDIX V

Guidance – Body Condition Scoring in Elephants
-Overview Approach-

University of Zurich[UZH]



Body condition score:	**Example Picture:**	**Criteria:**
0		extremely emaciated; thinner than BCS 1
1		ribs very prominent, more than 5 ribs can be counted easily; pectoral and pelvic bones very prominent, well defined edges of the scapula and ilium; vertebral column very prominent, deep depression alongside
2		ribs visible and up to 5 of them can be counted easily; pectoral and pelvic bones prominent, well defined edges of the scapula and ilium; vertebral column very prominent, depression alongside at the anterior end behind the scapula somewhat filled

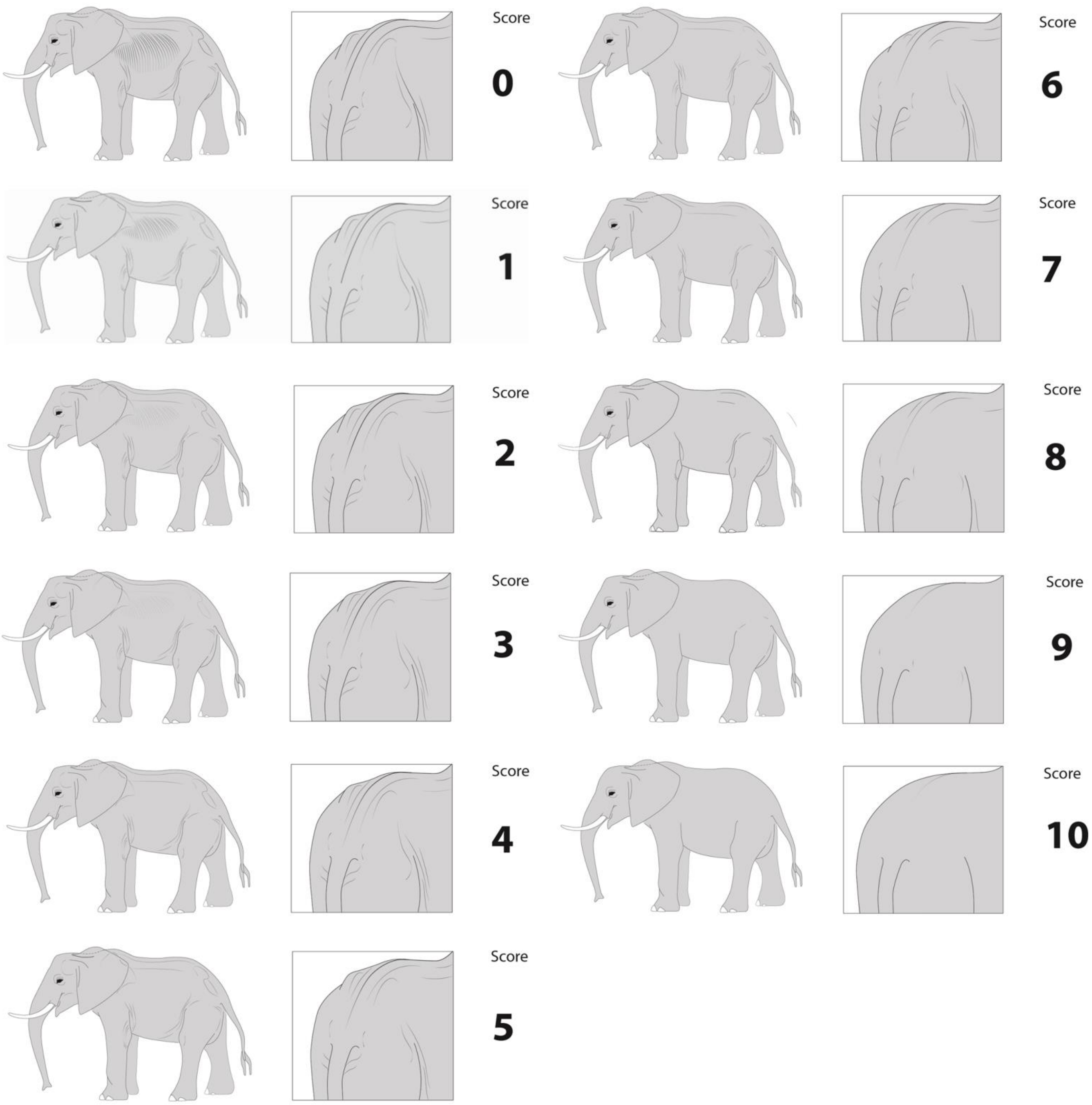
3		one or two ribs still prominent; pelvic bones can be seen and the anterior margin of the ilium is prominent; posterior margin of scapula can be seen; vertebral column prominent, depression alongside at the anterior end behind the scapula almost filled
4		no ribs are seen; scapula and pelvic bone visible with depressions at the posterior edge of the scapula and at the anterior edge of the ilium; vertebral column prominent
5		ribs not visible; scapula and pelvic bone can be seen with depressions at the posterior edge of the scapula and at the anterior edge of the ilium
6		pectoral and pelvic bones can be seen but the edges cannot be demarcated properly, no depression at the posterior edge of the scapula or the anterior edge of the ilium

According to recent findings by Norkaew et al. (2018) a BCS of 7-10 indicates an increased risk for alterations in metabolic markers and serum lipid profile. These alterations are considered to negatively affect health status.

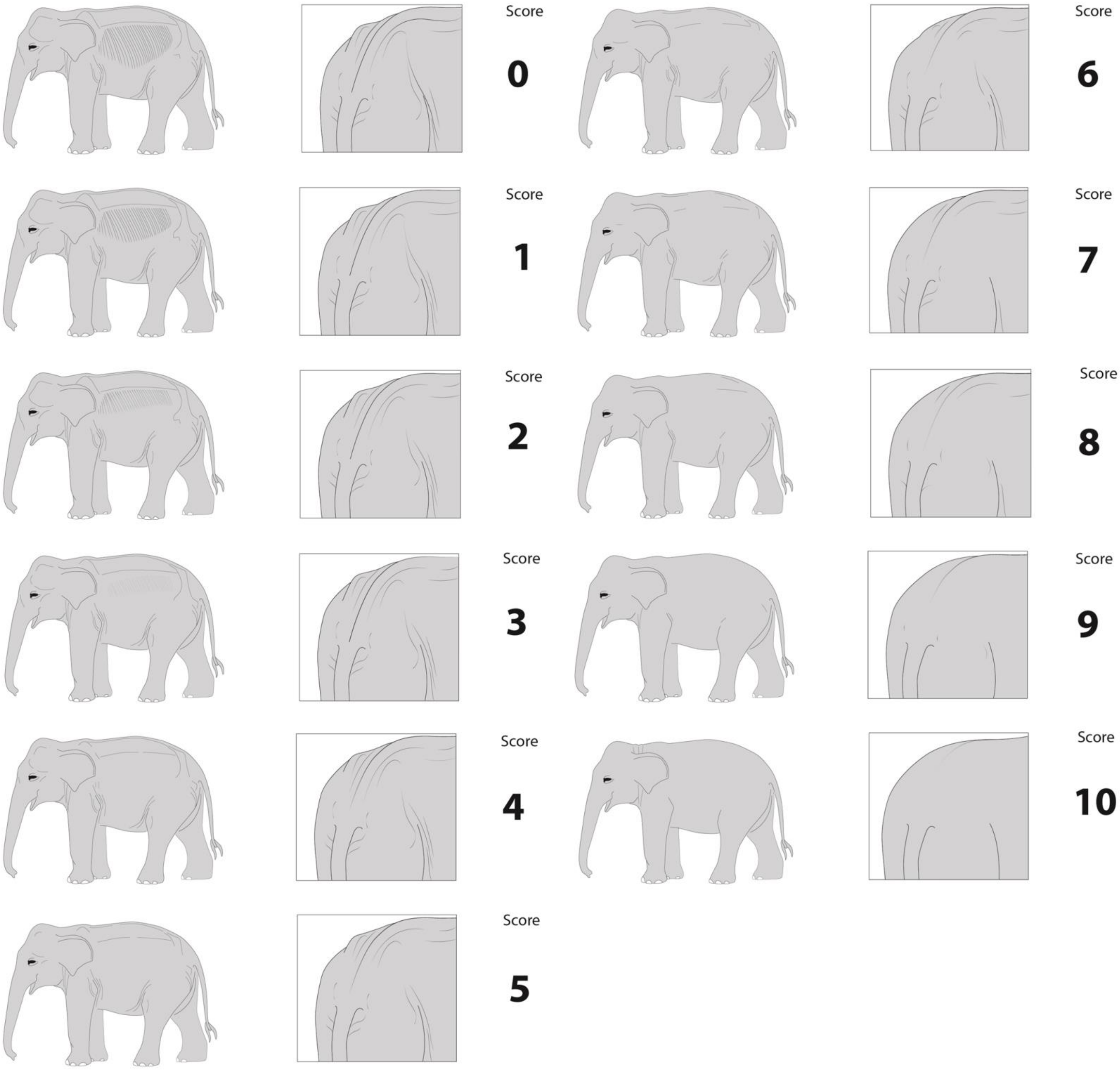
7		neither pectoral nor pelvic bones prominent, only visible when the elephant walks; vertebral column slightly apparent along the body, backbone visible as a ridge
8		no bone structures are visible even when the elephant is moving; thick skin folds are seen under the neck
9		no bone structures are visible even when the elephant is moving; back rounded; fatter than BCS 8; very thick, three to four centimeters of skin folds appear while walking in angle between foreleg and body and below neck
10		extremely abnormal obese; very thick rolls of skin fold below neck, which may measure up to five centimeters

Modified from Fernando et al. (2009), Morfeld et al. (2014) and Wijeyamohan et al. (2014).

Scoring protocol for the African elephant (*Loxodonta africana*)



Scoring protocol for the Asian elephant (*Elephas maximus*)



APPENDIX VI

Advice MVA vaccination in elephants March 2018

On request of the TAG-chair we herewith present a short note about the need to vaccinate Asian and African elephants against cowpox virus infection.

Cowpox (=orthopox) virus infections have been reported in both Asian and African elephants. Most cases have occurred in Western Europe. Symptoms may vary from minor lesions to fatal infection. Severe cases have been reported more frequently in Asian elephants than in African elephants, but both species are susceptible.

The European Association of Zoo and Wildlife Veterinarians has produced a fact sheet about cowpox infections in zoo animals, including several references to cowpox infections in elephants (Transmissible Diseases Handbook 2010, see attachment 1).

Prevention: preventive vaccination of African and Asian elephants is strongly recommended by the EAZWV and encouraged in the coordinated EAZA breeding programs of both species. The only vaccine available is Modified Vaccinia Ankara (MVA), which is produced and distributed by the Institut für Infektionsmedizin und Zoonosen, Dr. Robert Fux (**Robert.fux@lmu.de**), Veterinärstrasse 13, 80539 München, Germany. An example of a batch protocol provided by the producer of the vaccine is given in attachment 2. MVA has been used for many years in elephants, rhino's and tapirs without any side effects. Primo-vaccination of keepers or other staff members in contact with vaccinated animals is not required.

The recommendation for vaccination is:

First injection injections (s.c. or i.m.) of 4 ml MVA at the age of 12-16 weeks.
Second injection injections (s.c. or i.m.) of 4 ml MVA 4 weeks after the first injection. The producer of the vaccine advices and offers titer measurement before the vaccination and 3-4 weeks after second vaccination). In young and untrained elephants this may be not possible, and vaccination should be practiced without titer control.
Booster vaccinations: generally once every 2-3 years, depending on the titer.
Vaccination during pregnancy: following this vaccination advice, elephants should be immune before they become pregnant. There are no sound studies about the possible side effects of vaccination on the fetus. New non-vaccinated imports or elephants with unknown vaccination status should not be bred before they are properly vaccinated.

References: see Infectious Diseases Fact sheet (attachment 1)

Remarks: for ordering and using this noncommercial vaccine you need a special permit from your official veterinarians. Please contact your official veterinarian for further instructions regarding the import of MVA from Germany.
Some countries may impose restrictions regarding the contact between humans (staff, visitors) and elephants for the first two weeks after vaccination. However, there is no recommendation for this measure and it is not supported by scientific evidence nor by the producer. As MVA only replicates once in mammalian tissues it is safe and developed for use in humans.

Veterinary Advisors Elephant TAG:
Willem Schaftenaar, Thomas Hildebrandt, Michael Flügger, Endre Sos

APPENDIX VII

TB testing in EAZA captive elephants in the Elephant TAG

30th November 2018

Introduction

Tuberculosis (TB) is caused by bacteria in the genus *Mycobacterium*. Mycobacteria infect a broad range of species (mammals, birds, reptiles, amphibians and fish). Species susceptibility to specific Mycobacteria varies. In mammals, the term "tuberculosis" is used to define disease caused by *Mycobacterium tuberculosis*-complex organisms (*M. tb-complex*), which include *M. tuberculosis, M. bovis*, *M. africanum*, *M. microti, M. canetti, M. caprae, M. pinnipedii, M orygis and M mungi*. *M.tuberculosis*-complex has a zoonotic potential to elephant keepers, other zoo staff in contact with elephants and zoo visitors.
Mycobacterium tuberculosis is the predominant infection-causing agent in elephants although cases caused by *M. bovis* have occurred. *Mycobacterium szulgai*, an uncommon non-tuberculous Mycobacterium species, was associated with fatal disease in two African elephants and *Mycobacterium elephantis*, a rapidly growing Mycobacterium, was isolated from a lung abscess of an elephant that died of chronic respiratory disease. *Mycobacterium avium* is commonly isolated from elephants and is not generally associated with disease although a single fatal case has been reported (e.g. *Mycobacterium avium ssp. hominissuis* in an African elephant).

TB testing in elephants is a concern for all elephant keeping institutions. In Europe, no validated immunological tests for elephants, approved by EU-authorities are available at this time. However, veterinary authorities may request elephants to be tested on TB when they are moved to other countries. Zoos that receive elephants must be well aware of the risk of the import of TB in their collection. This makes it very important to build up a well documented history of elephant herds, including regular testing of elephants and monitoring other animals and personnel.
In the recommendations of the European Association of Zoo and Wildlife Veterinarians (EAZWV) for the approval of zoos according to the EU Directive 92/65, the trunk wash procedure for regular testing on tuberculosis (TB) has been discussed. In the final recommendations, a more general text was chosen (see point C.5.h.): "Specific guidelines for the systematic testing of specific animal species may be developed and recommended by the Infectious Diseases Working Group of EAZWV". This means, that at this time, no official guidelines for TB-testing of elephants are described under the BALAI directive.
However, this does not mean that zoos keeping elephants should refrain from establishing a routine testing protocol. The risk of introducing TB in a zoo through elephants is quite realistic as we can see from cases in the past decades both in the USA as well as in Europe. This document does not touch the issue of introduction of TB through other animal species and human contact, but should leave no doubts that a complete TB-surveillance is the only way to reduce the risks of TB in a zoo.
Every national or regional government may have its own interpretation about how to deal with suspected TB-cases. Much depends on the relationship between the zoo veterinarian and the official veterinary authorities. Generally, it helps if the zoo has a clear policy regarding its health surveillance system. Zoos that make all efforts to be certified according to the EU Directive 92/65 should have the intrinsic desire to stay free of infectious diseases like TB.

This document describes methods that can be used to collect information about the TB-status of individual elephants and an elephant herd. This document can only help to convince institutions that keep elephants to use all means available to minimize the risk of contracting and spreading tuberculosis in their elephants and personnel. Finally, it may help decision making in case of a planned elephant transfer; the reliability of a "TB-history report" depends a priori on the amount of data collected over the years, not only of a single test taken "on the day of transport".

Tools for TB-diagnosis in *live* elephants

A. <u>Bacteriological tests (culture and PCR of *M. tb complex)*</u>:

A positive culture and/or PCR confirms the presence of TB. Any positive culture should be confirmed by DNA-sequencing to trace the origin of the infection and exclude sample contamination at the lab. A negative result does not exclude infection and can implicate either: a truly negative animal, a TB-positive animal, but not yet shedding (closed TB, latency), or a TB-positive animal, but shedding low numbers of bacteria (open TB).

Samples to be taken from elephants for culture and PCR can be obtained by one of the following methods:

<u>Trunk wash (TW):</u>

The trunk wash procedure is an active manipulation at the elephant trunk, which can be performed in free and protected contact systems in non-immobilized elephants after they are conditioned for this procedure. The principle is that a sterile 0,9% saline solution (approx. 100 ml) is injected in each nostril of the trunk. The trunk has to be lifted actively by the elephant or passively by the keeper so that the solution is running up to the base of the trunk. The mixture of the solution and trunk mucus is collected in sterile plastic bags by active blowing of the elephant through its trunk (training required). The staff should protect themselves against spilling trunk content into their face. A full trunk wash procedure requires 3 different trunk washes performed within a period of 7 days. Each sample must be sealed and stored at 4°C. Depending on the quality of the samples, the diagnostic lab can decide to pool the samples for culture/PCR. Samples must be shipped to the TB-diagnostic lab immediately after the 3-rd sample has been taken. The maximum storage period at 4°C is 7 days. NB: follow the EU guideline for shipment of potentially hazardous biomaterials.

Trunk wash in a non-contact situation requires a full anesthesia of the elephant and a portable fluid pump and sucking system, which allows the operation under sterile condition. The external pump and sucking system will be connected to a sterile PVC tube (1 cm diameter, with distance markers) with a length of approx. 2 meter. The amount of sterile solution and the collection bag are like described before. In non-contact situations, a bronchoalveolar lavage (BAL) under standing sedation is the preferred procedure (see below).

<u>Bronchoalveolar lavage:</u>

The BAL can be performed under standing sedation. Two methods for approaching the deeper bronchi are being practiced currently.

(1) The BAL can be performed through the trunk. In addition to the sedation, a local block anesthesia in the trunk base is required in order to get relaxation of the cartilage "valves" present in the trunk base. This procedure requires a 5 m flexible endoscope.
(2) Another approach for the BAL is through the mouth. A mouth gag is required to protect the arm of the veterinarian who carries out the procedure. A 3.5 m long flexible endoscope can be advanced deep into the trachea, guided by the finger tips of the operator.

BAL allows visualization of the major bronchi. Different samples are taken by injecting 100-150 ml sterile 0,9% saline solution in different bronchi through a disposable catheter. Fluid is recovered by using a suction pump or 60 ml syringes. A typical sample contains watery fluid with some mucous material and air bubbles. The oral approach can also be used for obtaining a gastric lavage sample by advancing a flexible tube into the esophagus without using an endoscope). Such an additional sample increases the chance to detect mycobacteria originating from swallowed sputum and can also be used for culture and PCR.

Culture of suspected material should be performed at the National Veterinary Laboratory of the EU-member state. A final test result for culture may take up to 4 months. PCR-results are usually obtained within a few days. PCR should always be combined with culture.

B. Immunological tests

A positive immunological test confirms a prior contact of the animal's immune system with mycobacterial antigens. This may indicate either a TB-positive animal with active infection, a prior contact with TB, which lead to sterile immunity (no TB infection present), or a false-positive reaction due to contact with closely related non-pathogenic mycobacteria.
A negative result does not exclude infection and can implicate either: a truly negative animal, a TB-positive animal in which an immune response has not (yet) developed (closed TB, latency), or a TB-positive animal with advanced stage anergy (immunological non-responsiveness) (late stage clinical TB).
Immunological tests highly depend on the quality of the antigens used to read out the immunological reaction.

Tests to measure cell-mediated immune responses to *M. tb complex*:

- **Interferon-gamma (IFN-γ) test**: developed at the Veterinary Faculty in Utrecht. Like other diagnostic immunological TB-tests for elephants this test cannot be validated properly, because it is not possible to get enough samples from proven TB-positive and it is impossible to identify live proven TB-negative elephants. After stimulation of leucocytes with positive and negative controls, PPD-B, PPD-A and MTB-Complex specific recombinant antigens, the in vitro production of IFN-γ is measured using an elephant IFN-γ specific ELISA. Heparinized blood has to be delivered at the lab within 8 hours after collection. Cross reaction with antigens from related Mycobacterium spp. may cause a "false" positive test result. The interpretation of this promising test is still very difficult, but it is considered to contribute to the understanding of the immunological reaction of the elephant. At this moment the test can only be performed after making arrangements with the Div of Immunology, Dept of Inf Dis and Immunology, Fac of Veterinary Medicine, Utrecht University, Yalelaan 1, 3584 CL Utrecht, The Netherlands (Please contact Prof. Victor Rutten, v.rutten@uu.nl). If the time for transporting the sample to

Utrecht (NL) exceeds 8 hours, in consultation with Victor Rutten a regional TB-diagnostic lab can be approached to perform the first step in preparing the blood sample for conservation during prolonged transport.

- **Comparative skin test:** this "classical" test uses PPD-derived M.bovis-tuberculin and PPD-derived M.avium-tuberculin that is obtained from the National Veterinary Institute in each EU-country. Due to the special skin properties of elephants the use of the comparative skin test in this species has no diagnostic value. There is some evidence, that repetitive skin tests can booster the immune response in TB-positive animals. To measure this booster-effect a heparin blood sample must be taken 2-3 weeks after tuberculination (cells to be used for the IFN- γ, plasma for antibodies). However, more data are needed to confirm this phenomenon.

Tests to measure humoral immunity against *M.tb complex*:

In the past years several tests have been recommended and used, but the diagnostic value of measuring antibodies for tuberculosis in elephants using those tests has proven to be questionable. Several **non-validated ELISA's** have been routinely available at the Central Veterinary Institute Lelystad (Netherlands). Antigens that were used: M.bovis crude antigens, M.avium crude antigens, and recombinant MPB70. However, in spring 2018 (after a validation test of the ELISA's performed in 284 elephant samples) the CVI-Lelystad announced that the routine testing has been discontinued, as the sensitivity of the test is too low to use it as a predictive test for individual elephants. For specific situations (i.g. for screening a TB-confirmed herd), CVI-Lelystad is developing new ELISA's. (Address: Wageningen Bioveterinary Research, Houtribweg 39, 8221 RA Lelystad, the Netherlands).

The formerly used Elephant TB STAT-PAK Assay and MAPIA (Chembio, USA) are no longer available for Europe. TB STAT-PAK has been replaced by the **Elephant DPP-VetTB test**; this test has been used in the past years (USA and Europe) and has been subject of many debates because of the high number of (probably) false-positive results in assumed negative elephants and negative results in confirmed positive elephants. The availability of this test in Europe has been and still is unpredictable. At this moment the vet advisors of the European Elephant TAG do not recommend its use on a routine basis as interpretation of the high number of (possibly) false-positive results caused a lot of confusion and conflict with veterinarian authorities and participants of the breeding programs formerly.

Monitoring the TB status of elephants in zoos

The recommended way to monitor the TB status in individual elephants and in the elephant herd is based on:

1. History of TB in that particular zoo, including other mammalian species.
2. Annual trunk washes or tracheal washes performed in all elephants of 4 years and older followed by culture and PCR. This procedure includes 3 trunk washes performed in one week (non-sedated animal) or 1 bronchoalveolar lavage (standing sedation). Each trunk wash sample must be submitted as an individual sample for culture and PCR.
3. Full necropsy of every dead elephant.

The EEP-coordinator sends out an annual survey in which culture results should be provided.

Pre-transport testing

When an elephant will move to another institution, the EEP-coordinator shall contact the exporting institution in order to collect relevant data about the history of the animal(s) and to make arrangements for the required diagnostic procedures.

1. A **report about the history** of the animal(s) that will be moved must be provided, including the following data:

• Species / ID / date of birth / location of birth
• Locations where the animal has been kept during its entire life, including dates of entry, known history of TB cases at these locations (endemic areas e.g.)
• Has the animal previously been suspected of tuberculosis or treated for this disease?
• Has there been any known direct or indirect contact with confirmed or suspected TB cases in herd mates or other mammalian species, including humans?
• List of data when blood was sampled, tested and stored at below -20°C
• All results of TB-tests performed in the animal and in the herd in the past 5 years
• Does the animal show clinical signs that are suggestive for tuberculosis?

2. **TB testing:** Each elephant that will be moved to another institution must be subjected to 3 trunk washes performed in 1 week or 1 bronchoalveolar lavage (BAL), 4 months* prior to transport (culture and PCR). At the same time, an Interferon-gamma test (IGRA) must be performed. Subsequently, the culture and PCR procedure must be repeated 2 months* prior to transport. Each trunk wash sample must be submitted as an individual sample for culture and PCR.

   NB: BAL should always replace the trunk wash in case of:
   a. Unexplained chronic weight loss
   b. Any positive result from a serological test (IGRA and/or DPP)

   ** The standard culture period for Mycobacterium sp. may differ per country. If the reference lab in the country of the sender requires a culture period longer than 2 months, the culture and PCR-procedures should be performed with a 2 months interval, scheduled in such a way that the results of the last procedure are received not more than 2 weeks prior to the expected transport.*

The animal history and the TB-test results are communicated to the EEP-coordinator, who in turn sends the results to the veterinary advisors of the European Elephant TAG. In case of doubt, the veterinary advisors will consult specialists in the field and provide a final decision about the transport.

Mtb-complex exposure Risk Categories for Elephants

Elephants are placed into one of four groups depending on their risk of exposing the environment with Mtb-complex. Testing requirements for elephants vary according to what risk group they belong to. Note that these groups are determined by two factors: herd history and a positive trunk wash/BAL culture, but not primarily by immunological test results. The veterinary advisor for the TAG can recommend increasing the amount and type of testing for any elephant based on the concerns and history of the animals involved.

Category A elephants:

Risk of infection with Mtb-complex: Low
Known exposure to an Mtb-complex culture/PCR-positive animal: None within the past five years.
Test history: Negative for past five years (or shorter in animals under the age of 8 years) on annual triple TW testing/BAL by culture/PCR.
Recommended testing: Routine. Triple TW culture technique or single BAL done minimally annually.
Travel restrictions: None.

Category B elephants:

Risk of infection with Mtb complex: doubtful
Known exposure to an Mtb-complex culture/PCR-positive animal: Exposure to a Mtb culture-positive elephant, keeper or another mammal has occurred within the past five years.
Test history: negative test results of the previous 5 years have no consequences for the actual status.
Recommended testing: single BAL and IGRA should be performed immediately after confirmed TB (Mtb-complex) in a herd member, a keeper or another mammal. This is followed by quarterly triple TW for one year. After 12 month a single BAL and IGRA should be performed. If all culture/PCR tests remain negative, these elephants return to Category A status. If culture/PCR are positive (including sequencing of the Mycobacterium sp.), these elephants go to Category C. All test results should be communicated with the vet advisor-group.
According to the guidelines of the national authorities regarding confirmed TB-infection: proper hygiene measures must be implemented, including medical control of staff members with access to the elephant facility, proper disposal of animal excretions, species-specific TB-testing of all animals in close contact with the elephants (mixed exhibits), disinfection of the exhibit (inside and outside).
If a Category D elephant (see below) is present at the institution, the B-status will apply to all other herd members as long as this Category D elephant is present at the institution plus the required period for the testing protocol as described above.
Travel restrictions: no travelling neither receiving elephants from other institution.

Category C elephants:

Risk of infection with Mtb-complex: high.
These elephants are positive for Mtb-complex on PCR or culture, including DNA-sequencing. An action plan following a positive test result should be discussed immediately with the vet advisor-group.
Depending on the national legislation and the Mycobacterium subspecies, the options for category C animals are:

- Treatment: based on the antibiogram of the Mycobacterium. These elephants go to Category D.
- Euthanasia in case:
    - treatment is not allowed by the national authorities;
    - Mycobacterium strain(s) is (are) not sensitive for any of the suitable antibiotic compounds;
    - treatment protocol cannot be fulfilled for the recommended treatment time;

  - this is the zoo's decision.

Travel restrictions: no travelling neither receiving elephants from other institution.

Category D elephants:

Risk of infection with Mtb-complex: unknown to high.

These elephants are treated elephants that belonged to Category C.

Recommendations: annual monitoring the treatment efficacy by BAL and IGRA for the rest of its life for staff safety reasons. Whatever the success of the treatment and the test results are, these elephants can never be considered as TB-free animals.

Travel restrictions: only to zoos with Category D elephants

Notifications of an Mtb-complex culture positive elephant

After an elephant tests positive for Mtb-complex via culture and DNA-sequencing derived from TW/BAL or any other body fluids (NB uterine discharge and faeces!), the diagnostic testing laboratory will contact the official veterinarian and the attending veterinarian. Once a positive culture is received, the elephant is considered infected with Mtb-complex, and the following notifications and steps are necessary:

1. Regulatory personnel such as:
   - State Veterinarian
   - State/local Public Health officials
   - TAG vet advisor-group
   - Species coordinator
2. Facility personnel including:
   - All staff working with the elephant including barn staff, veterinary staff and volunteers
   - Upper management & legal teams
   - Public relations, marketing, and communications teams
   - Human resources (for TB-testing the staff)
3. Notification of other facilities where the Mtb-complex culture-positive elephant has been in the past.
4. Discussions regarding safe elephant handling, use of personal protective equipment (PPE) such as N-95 respirators, and barn cleaning protocols to prevent zoonotic transmission or spread to other elephants in the herd should occur in collaboration with regulatory and facility personnel.
5. Mixed exhibit: animals sharing the exhibit with a TB-positive elephant should be considered as potential TB-carriers and tested and handled accordingly.

Summary: What to do when tuberculosis has been confirmed in an elephant?

In the unfortunate event that tuberculosis has been confirmed either during necropsy or from culture or PCR of samples taken from a living elephant, the official authorities should be informed. Although not explicitly mentioned under BALAI, *Mycobacterium bovis* is a notifiable disease when confirmed in *Mammalia*, in particular *Antilocapridae, Bovidae, Camelidae, Cervidae, Giraffidae*, and *Tragulidae* (Annex A to Directive 92/65/EEC).

Though elephants are not specified as such, potentially close contact with the above mentioned species and the close contact with humans justify the close cooperation with official veterinary and human medical authorities. The zoo veterinarian should form a small group of experts, including a representative of these authorities, in order to make a surveillance and monitoring program. This plan should include (1) screening of zoo staff and individuals that have been in close contact with the affected elephant, (2) screening of contact elephants as well as other species sharing the same enclosure, (3) informing the zoo where the elephant has come from and (4) informing any zoo that has received contact animals during the period that shedding may have taken place. Treatment of TB-confirmed or suspected elephants is an option to be discussed with the zoo staff as well as with the official authorities. One should be aware of the fact that there is evidence that even after intensive treatment of some elephants, shedding has reoccurred in these animals after several years (see category D). Some countries may not allow treatment of confirmed or suspected cases. Screening of contact elephants follows the methods described in this document (see category B).

In addition to the TAG elephant necropsy protocol, the following recommendations should be taken into account when TB is suspected in an elephant.

Wear a mouth and nose protector when necropsy is performed in case of a TB-suspect case and work with a minimal staff at the site of necropsy.
All elephants undergoing necropsies should have a careful examination of:

- The tonsillar regions and submandibular lymph nodes for tuberculous appearing lesions. Take any nodes that appear caseous or granulomatous for culture (samples should be shipped for culture instantly at 4°C according to EU-legislation for shipment of hazardous biomaterials), and a separate part for histology (fixation in buffered 10% formalin). Back-up samples should be stored preferably at -80°C.
- Split the trunk from the tip to its insertion and take samples of any plaques, nodules or suspicious areas for TB diagnosis as above. Look for and collect possible extra-thoracic TB lesions, particularly if there is evidence of advanced pulmonary TB.
- Open the trachea and look for nodules or plaques and process as above. Regional tracheal lymph nodes should also be examined and processed accordingly. Take samples of any suspicious lesions. The thoracic organs carefully for early stages of TB as follows: after removal of the lungs and trachea, locate the bronchial nodes at the junction of the bronchi from the trachea. Use clean or sterile instruments to section the nodes. Freeze half of the lymph node and submit for TB culture (even if no lesions are evident). Submit sections in formalin for histopathology. Carefully palpate the lobes of both lungs from the apices to the caudal borders to detect any firm (nodular size) lesions. As the pleura in elephants are fused, the lungs are firmly attached to the thoracic wall. This makes handling of the elephant lungs more difficult than in other species. Regional thoracic lymph nodes should also be examined and processed accordingly. Take samples of any suspicious lesions.

In case of any suspicion of TB in an elephant the Veterinary advisor-group of the elephant TAG must be informed immediately:

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APPENDIX VIII

ELEPHANT NECROPSY PROTOCOL

(Based on the AAZV – SSP protocol)

INTRODUCTION

The purpose of this protocol is to provide a format for the systematic collection of information and samples that will add to our knowledge of elephants. All European zoos holding elephants registered in the EEP will receive a copy.

The protocol consists of 2 parts. Part one gives some guidelines for the necropsy procedure. Part two is the actual PM report. You are kindly asked to fill in part two on the computer and return it to the addresses indicated.

PART 1

EQUIPMENT CHECKLIST

1. Standard large animal necropsy instruments. Multiple scalpel handles, duplicates or triplicates of other instruments. Extra box of scalpel blades, knife sharpener, and a continual supply of sharp knives.
2. Retractors of various sizes and shapes. Self-retaining retractors with one or two movable arms mounted on a slide bar are most useful.
3. Sterile instruments for culture collection.
4. 10% neutral buffered formalin.
5. 4% buffered glutaraldehyde
6. Containers for sample collection.
7. Culture swabs, sterile urine cups, glass slides.
8. Serum tubes for blood and urine collection.
9. Aluminum foil and plastic bags for freezing tissues.
10. Labels and waterproof marking pens.
11. Scale for obtaining organ weights.
12. Tape measure, at least 2 meters long.
13. Chain saw, axe, or reciprocating saw to cut through the cranium. Hammers, chisels and handsaws.
14. Hoist/crane.
15. Carts on rollers to move heavy parts.
16. Coveralls, boots, gloves, caps, masks, protective eye and head gear.
17. Accessible water supply with hose.
18. Camera and film, extra batteries.
19. First aid kit.
20. Surgical masks approved for TB exposure.

LOGISTICS AND NECROPSY TIPS

Heavy equipment may be necessary to move a dead elephant. For an on site necropsy, chains and a tow truck may be sufficient to reposition the animal or to move it a short distance. If the animal must be transported to a remote site, a truck with a hoist will be needed. It may be easier to manipulate the animal onto a flatbed trailer. Vehicles must be able to handle these approximate weights: female Asian: 2,300 - 3,700 kg; male Asian: 3,700 - 4,500 kg; female African: 2,300 - 4,000 kg; male African: 4,100 - 5,000 kg. If transportation will be delayed, the carcass can be covered with ice.

If death is imminent or euthanasia is planned, completion of the measurement checklist ante mortem will save time at necropsy. Otherwise, measurements should be done as soon after death as possible.

Assigning specific tasks to team members will help the necropsy to proceed in an orderly manner. For example, a team may be assigned to each of these areas: head, forelegs, hind legs, abdominal region. One person should oversee the collection, labelling, and processing of research materials and any communication concerning research requests. It may be helpful to designate a media spokesperson.

Dissection of the head is best completed after separating it from the body. A good portion of the cranium must be damaged to remove the brain intact; a chain saw, large axe, and chisels are needed to penetrate the thick cranium. A battery operated reciprocating saw with a replaceable metal cutting blade may be safer and easier to handle. A posterior approach to brain removal can be made by 3 connecting deep cuts with a chain saw in the margins of the flattened triangle formed at the base of the elephant skull. Then remove the bony plate in chunks with a curved crow-bar. Use of a chain saw on bone can be hazardous and cause shrapnel-like fragments to be launched. Protective head and face gear should be worn by the chain saw operator and personnel in the immediate area.

Dissection of the thoracic cavity is best performed by at least two people. After the initial incision at the ventral midline is made, one person holds the retractor and the other cuts the tensed skin. Once the sternum is exposed, the ribs are separated at the cartilaginous attachment and adjustable retractors are applied to hold the cavity open. The heart, lungs, and associated structures may be removed "en bloc" with the diaphragm. Visceral and parietal pleura are normally adhered; there is little pleural space.

Laboratory Studies: Please include results of cytology, fluid analysis, urinalysis, serum chemistries, bacteriology, mycology, virology, parasitology, X-ray, photographs, or other data collected.

TISSUE CHECK LIST

Freeze 3-5 cm blocks of tissue from lesions and major organs (e.g., lung, liver, kidney, spleen) in small plastic bags. Freezing at -70 degrees Celsius in an ultra-low freezer is preferred. If this is unavailable, freezing at conventional temperatures is acceptable (use a freezer without an automatic defrost cycle if possible).

Any lesions noted in the lungs should be submitted for mycobacterial culture. Bronchial lymph nodes should be cultured for TB even if normal in appearance. Preserve as many of the tissues listed below as possible in 10% buffered formalin at a ratio of approximately 1 part tissue to 10 parts solution. Tissues should be no thicker than 0.5 to 1.0 cm. Fix diced (1x1 mm) pieces of kidney, liver, spleen and lung in a suitable EM fixative if possible - glutaraldehyde base e.g., Trump-McDowell fixative. NOTE: There is generally no need to fix and label each tissue separately. Take 2 sets of fixed tissue. Bank one set. Also, freeze post mortem serum (from heart), urine and any abnormal fluid accumulations.

Checklist

Adrenal	Kidney	Penis	Thymus
Blood *	Large intestine	Pituitary	Tongue
Bone with marrow	Liver	Prostate	Trachea
Bulbo-urethral gland	Lung	Salivary gland	Trunk cross section
Brain	Lymph node	Seminal vesicles	Uterus/cervix
Coecum	Mammary gland	Skin	Ureter
Diaphragm	Muscle	Small intestine	Urinary bladder
Oesophagus	Nerve (sciatic)	Spinal cord	Vaginal/urogenital canal
Eye	Ovary/testis	Spleen	
Heart/aorta	Pancreas	Stomach	
Haemal node	Parathyroid	Temporal gland	

***** Collect post mortem blood, separate serum and freeze for retrospective studies.

PART 2. GROSS EXAMINATION WORKSHEET

Institution/Owner	
Address	

Species	ISIS:	Studbook:
Name:		
Birth date/ age	Sex:	Captive born ☐ Wild caught ☐

Death date		
Death location		
Necropsy date		
Necropsy location		
Post mortem Interval		
Weight (kg)		Actual ☐ Estimate☐
History (clinical signs, circumstances of death, clinical lab work, diet & housing)		

GROSS EXAMINATION

If no abnormalities are noted or if the organs were not examined, mark as "normal" or "NE" (not examined) respectively.

General examination (physical and nutritional condition, skin, body orifices, superficial lymph nodes):

Musculoskeletal System (bones, marrow, joints, muscles):

Body Cavities (fat stores, pleura, thymus, lymph nodes):

Spleen	

Respiratory System (trunk passages, pharynx, larynx, trachea, bronchi, lungs, regional lymph nodes; submit lung lesions for TB culture; bronchial lymph nodes should be cultured for TB even if normal in appearance):

Cardiovascular System (heart, pericardial sac, great vessels, myocardium, valves, chambers):

Digestive System (mouth, teeth, tongue, oesophagus, stomach, small intestine, coecum, large intestine, rectum, liver, pancreas, mesenteric lymph nodes):

Urinary System (kidneys, ureters, bladder, urethra):

Reproductive System (testes/ovaries, uterus & cervix, penis/vagina, urogenital canal, prostate, seminal vesicles, bulbo-urethral gland, mammary gland, placenta):
Endocrine System (thyroids, parathyroids, adrenals, pituitary):

Central Nervous System (brain, meninges, spinal cord):

Sensory Organs (eyes, ears):

Additional Comments or Observations:

Final Pathology report including microbiology, immunology, histology and other test:

Primary Pathologist:

Name:

Lab:

Address:

Phone:

E-mail:

Please send this report by e-mail to: W.Schaftenaar@Rotterdamzoo.nl

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APPENDIX IX

Quality of Life Assessment for Elephants

Elephant´s name:	**Species:**
Age:	**Date of assessment:**

1) Pain assessment and treatment options

a) Is the elephant exhibiting any behaviors indicative of pain (lethargy, changes to gait, trunk sucking, head pressing etc.)?

b) If not behavioral obvious, is there a reasonable suspicion of pain (e.g. radiographs indicating osteoarthritis, historical information regarding disease or injury etc.)?

c) How long have these the above been exhibited? What has been the frequency? Has the frequency or intensity changed over time?

d) Is the elephant currently being treated for the pain? If yes, with what and for how long? If no, can the pain be treated?

e) What accommodations have been made (e.g. changes to operational procedures, changes to training or husbandry, addition of softer substrates, habitat access etc.)?

Scoring Guidelines

0 – No obvious discernable pain.

1 – Intermittent recurring pain managed satisfactorily with medication, treatment, or accommodations.

2 – Chronic pain managed satisfactorily with medication, treatment, or accommodations.

3 – Chronic pain moderately or intermittently managed with medication, treatment, or accommodations.

4 – Chronic pain unsatisfactorily managed with medication, treatment, or accommodations.

Score: ……………

2) Medical condition and treatment options

a) What is the veterinary medical prognosis for the elephant – can current health alterations be treated and if so, completely or satisfactorily resolved?

b) If medical treatment is possible, how prolonged and stressful will it be for the elephant? What is the likelihood of success?

c) Considering life expectancy and the normal lifespan, how close to this age is the elephant?

Scoring Guidelines

0 – No known medical condition/no treatment required.

1 – The elephant is receiving medications which are satisfactorily treating the condition, or condition has high probability of resolution with treatment.

2 – The elephant is receiving medications which are moderately treating the condition, or condition has moderate probability of resolution with treatment.

3 – The elephant is receiving medications which are moderately treating the condition, but condition has no chance of resolution with treatment. Further treatment options may be available, but have not been tried so far.

4 – The elephant is receiving medications which are moderately treating the condition, but condition has no chance of resolution with treatment. According to current knowledge, no further treatment options are available.

Score: ……………

3) Appetite and food consumption

a) Is the elephant´s appetite decreased? If yes, for all foods or some?

b) Is special food preparation or presentation required to ensure adequate consumption?

Scoring Guidelines

0 – Elephant is eating regular diet with little or no manipulation of preparation or presentation.

1 – Consuming 75-100% of normal diet with some manipulation, or consuming 50-75% with no manipulation.

2 – Consuming 50-75% of normal diet with some manipulation, or consuming less than 50% with no manipulation.

3 – Consuming less than 50% of normal diet with considerable manipulation, or eating only treats or special foods.

4 – Elephant is consuming less than 25% with considerable manipulation and is rarely taking treats or special foods.

Score: ……………

4) Physical condition

a) Is the elephant maintaining body condition within normal limits for this individual? What is the elephant´s current body condition score (BCS)? How did BCS in this individual develop over time?

b) Is the elephant maintaining body condition within normal limits for species and age? What is the condition of the skin?

c) What is the elephant´s most current body mass? What is the individual´s body mass? What is the percentage of difference (ideal body mass - current body mass/ideal body mass x 100 [%])?

d) How has body mass changed over time?

Scoring Guidelines

0 – BCS in the ideal range (depends on scoring protocol applied). Muscle tone is considered good for individual´s history and age. Skin is healthy, lacks dead skin buildup or dryness.

1 – According to BCS considered under- or overconditioned. Muscle tone slightly less than expected for individual´s age and history. Skin may be slightly dry or have minor minor buildup covering less than 25% of body surface.

2 – According to BCS considered under- or overconditioned. Muscle tone is diminished beyond what would be expected for individual´s age and history. Dead skin buildup covering 50% of body surface and/or skin condition is dry and ashy in over 50% of body surface.

3 – According to BCS the elephant is obese. Muscle tone is severely deteriorated. Dead skin build up covers most of body with poor skin condition over most of the body surface.

4 – According to BCS the elephant is emaciated. Muscle tone is severely deteriorated. Dead skin build up covers most of body with poor skin condition over most of the body surface.

Score: ……………

5) Mental and psychological health condition

a) Is the elephant able to maintain a near-normal behavioral repertoire and activity budget? Does the elephant engage in behaviors beyond basic maintenance (e.g. eating/drinking, mudding/dusting etc.)?

b) Does the elephant use and interact with enrichment?

c) Is the elephant participatory in training sessions? Does the elephant engage with caregivers?

d) Have there been changes to the elephant´s normal personality when engaged with enrichment/training/non-formal interactions with caregivers?

Scoring Guidelines

0 – The elephant participates in daily husbandry and management practices, engages with enrichment, participates in training sessions (within normal limits for this individual), and responds to caregivers in manner consistent with this individual´s behavior.

1 – The elephant engages in preferred activities with normal treats or without special accommodations, but is reluctant to participate in undesirable or less attractive activities without special treats or accommodations.

2 – The elephant engages in most activities only with special treats or accommodations and engages in less preferred activities less than 50% of the time even with special treats or accommodations.

3 – The elephant participates only in favored activities for special treats or with accommodations.

4 – Withdrawn, non-participatory, and barely acknowledges caregiver´s presence. Does not engage with enrichment and refuses to participate in training in over 90% of the time.

Score: ……………

6) Social condition

a) Is the elephant maintaining the position within the social group? Can the elephant move with the group and avoid abnormal displacement (dependent on the elephant´s position in the hierarchy)?

b) Is the elephant bonded with another elephant? Has there been a change in this relationship?

c) Is the elephant in a social situation? Is it full or part time? If part time, how much time? Is there visual, auditory, olfactory, tactile, full physical contact with other elephants?

d) How social is this individual? What is her social history?

e) Does case management require isolation? Long or short term isolation? How will the isolation affect quality of life?

f) Does treatment and therapy for this elephant affect herdmates?

g) Is the elephant interacting and socializing with caregivers within normal limits?

Scoring Guidelines

0 – Sociality within normal limits for this individual.

1 – Individual´s normal social activity is diminished, but the elephant is maintaining its position within the group. Interactions with humans are slightly decreased.

2 – A moderately social individual is unable or unwilling to engage in normal social activity with elephants and humans. Social isolation required for treatment causes minimal disruption or social stress to the individual and group.

3 – A highly social individual is engaged in social behavior at a rate of less than 50% the normal level. Social isolation required for treatment causes stress to the individual and group, but is short term.

4 – Social interaction has virtually halted with both elephants and caregivers. Long term social isolation is required for treatment.

Score: ……………

7) Mobility

a) Is the elephant alert and active? Can the elephant move in all spaces (barn, small yards and large habitats) normally? Can the elephant access normal areas of enclosures without significant risk of injury to self?

b) Is the elephant´s gait normal (within normal limits for individual)?

c) Are there changes to normal movement patterns (e.g. seasonal habitat use, foraging etc.)?

Scoring Guidelines

0 – Mobility and enclosure utilization within normal limits.

1 – Exhibits slight limp or gait change, but uses easy parts of enclosure in a close to normal manner. More challenging areas of the habitat are used less. Movements may be slower than normal.

2 – Refusal to use more challenging aspects of habitats. Shows considerable limp or gait changes and locomotion is considerably slowed.

3 – Significant changes to distance walked, habitat use, and any normal activities requiring locomotion. Use of habitats limited to easy terrain.

4 – Locomotion is reduced to very little movement. Refuses to leave a particular area (e.g. barn).

Score: ……………

8) Further aspects

a) Are there any other issues not covered above?

Scoring Guidelines

0 –

1 –

2 –

3 –

4 –

Score: ……………

Total score: ……………

Scoring evaluation

0-5	Very good quality of life.
6-11	Quality of life somewhat affected. Elephant should be closely monitored and accommodations immediately implemented.
12-17	Quality of life significantly diminished. New husbandry and treatment options must be developed and implemented.
18-23	Quality of life seriously compromised. Euthanasia may be considered if more aggressive treatment options are not available or feasible.
24-28	Very poor quality of life. Euthanasia should be considered.

Scoring evaluation if "*8) Further aspects*" is used

0-6	Very good quality of life.
7-13	Quality of life somewhat affected. Elephant should be closely monitored and accommodations immediately implemented.
14-20	Quality of life significantly diminished. New husbandry and treatment options must be developed and implemented.
21-27	Quality of life seriously compromised. Euthanasia may be considered if more aggressive treatment options are not available or feasible.
28-32	Very poor quality of life. Euthanasia should be considered.

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