EAZA Best Practice Guidelines

Black rhinoceros (*Diceros bicornis*)

Picture: Black rhino at Chester Zoo

Editors: Dr Mark Pilgrim and Rebecca Biddle MBiolSci., NEZS Chester Zoo

Contact information: Chester Zoo, Cedar House, Caughall Road, Chester, CH2 1LH, 01244 389 879

Name of TAG: Rhinoceros TAG

TAG Chair: Dr. Friederike von Houwald

Edition: 2

EAZA Best Practice Guidelines disclaimer

 The information contained in these EAZA Best Practice Guidelines has been obtained from numerous sources believed to be reliable. EAZA and the EAZA Rhino TAG make a diligent effort to provide a complete and accurate representation of the data in its reports, publications, and services. However, EAZA does not guarantee the accuracy, adequacy, or completeness of any information. EAZA disclaims all liability for errors or omissions that may exist and shall not be liable for any incidental, consequential, or other damages (whether resulting from negligence or otherwise) including, without limitation, exemplary damages or lost profits arising out of or in connection with the use of this publication. Because the technical information provided in the EAZA Best Practice Guidelines can easily be misread or misinterpreted unless properly analysed, 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 optimalfor 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.

Preamble

These Best Practice Guidelines were based on 'concept husbandry guidelines for Black rhino (*Diceros bicornis*)' which were produced by Valentijn Assenberg and Thijs van den Houten for the final thesis of their Animal Management course at the Van Hall Larenstein Institute.

The data to form the concept husbandry guidelines was collated using a literature study and a questionnaire. We are grateful to the following people who assisted by completing the questionnaire: Andreas Knieriem (Hannover Zoo), Gerd Nötzold (Leipzig Zoo), Jiri Hruby (Dvur KraloveZoo), Robert Zingg (Zurich Zoo), Ulrike Cyrus (Zurich Zoo) Helen Massey (NEZS Chester Zoo) and Xavier Vailliant (Pont-Scorff Zoo). The literature was chosen from a number of sources and a full reference list can be found at the end of this document.

The Veterinary Guidelines were writen by Jane Hopper (Aspinall Foundation Uk), Javier Lopez (Chester Zoo UK), Linda van Sonsbeek (Rotterdam Zoo NL), Julia Stagegaard (Ree Park Safari DK), Robert Hermes (IZW GER) and Marcus Clauss (University of Zurich, SW). Additionally we are gateful to Marcus Clauss and Jürgen Hummel for their work on the Nutrition Section, and Dr Sue Walker and Dr Katie Edwards for their additions regarding Black rhino endocrinology and its applications to captive management.

Citation

Pilgrim, M. & Biddle MBiolSci, R. (2020). EAZA Rhinoceros Taxon Advisory Group*, Best Practice Guidelines for the black rhinoceros (Diceros bicornis) – 2nd edition*. European Association of Zoos and Aquariums, Amsterdam, The Netherlands, 133pp.

DOI

10.61024/BPG2020BlackRhinocerosEN

Contents

Introduction

Once plentiful across Africa, the Black rhinoceros *Diceros bicornis* is now classified as Critically Endangered by the IUCN (IUCN Redlist, 2012). During the last 60 years the Black rhino population has declined by almost 90% reaching a low of 2,410 individuals in 1995. Since then and until recently numbers have slowly increased. This has been due to concerted conservation efforts to protect rhinos from poaching and to metapopulation management including founding or enhancing populations through translocation. At the end of 2012 the estimated number of Black rhinos left in the world was 5,055 individuals.

Diceros bicornis is the only species within its genus; there are four distinct subspecies recognised by IUCN/SCC African Rhino Specialist Group; The European Association of Zoos and Aquaria (EAZA) is working with two of the four subspecies (Eastern and Southern) and is managing them as distinct subpopulations. A viable European captive programme exists for Eastern Black rhinoceros (*D. b. michaeli*) and a non-viable European captive population exists for Southern Black rhinoceros (*D. b.*

minor) of 1.1 animals. These are planned to be repatriated to Africa. There are no South western (*D. b. bicornis*) in zoos nor are there any North western (*D. b. longipes*) Black rhinoceros, which recently became be extinct in the wild (IUCN 2011). Globally there are now 799 *D. b.michaeli,* making it the rarest of the three remaining Black rhino subspecies (IUCN 2012).

Due to the Critically Endangered status, Black rhinos in European Zoos are under the most intensive level of management, an EEP. The purpose of this programme is to secure a genetically healthy and sustainable captive population which may serve as a backup population for the wild. An international breeding programme was set up in 1966. This breeding programme contains an international studbook and manages the captive Black rhino population (Dollinger and Geser, 2008). The goals of this international breeding programme are self-sustaining reproduction, demographic security and stability, genetic diversity adequate for animal fitness and population adaptability and target population sizes sufficient to achieve these genetic and demographic goals (Foose and Wiese, 2006).

Worldwide there are 240 Black rhinos in zoos, including 64 in 15 EAZA zoos (2012) and another 15 in 2 non-EAZA zoo's. EAZA members have established Taxon Advisory Groups (TAGs) for different groups of animal species that are kept in zoos and aquariums. One of the main tasks of a TAG is to develop Regional Collection Plans that describe which species are recommended to be kept. The TAGs also identify which species need to be managed in a European breeding programme called EEP (European Endangered species Programme) (EAZA, 2008).

The mission of the EAZA Rhinoceros TAG is:

'*To ensure all captive populations are healthy, self-sustaining and genetically viable and are capable of being an effective tool in support of rhino conservation in the wild.*'

The goals of the EAZA Rhinoceros TAG are:

Population management

- To ensure each EEP population is self-sustaining and genetically viable in the long term.
- To ensure each taxon has ambitious targets for the retention of maximum gene diversity (~ 90% GD per century).
- To work more closely with other regions to support effective population management.
- To work to overcome obstacles which impinge upon population and genetic management goals; e.g. international transfers and importation of new founders.

Husbandry and welfare

- To ensure each EEP drives ongoing welfare and husbandry improvement.
- To ensure Best Practice Guidelines are in place for all EEPs by 2012 and reviewed at least every second year.
- To develop an audit process to ensure all holders are compliant with Best Practice Guidelines by 2015.

- To identify and support research priorities which advance husbandry and welfare and support the development of Best Practice Guidelines.

Education and research

- To ensure the captive populations provide a significant educational and research resource capable of contributing to rhino conservation.
- To recruit an education advisor to the TAG.
- To measure the impact of zoo based education specific to rhino conservation and assist in the improvement of zoo based education.
- To set up a research advisor team to the TAG.
- EEP coordinators to identify research priorities prioritising projects conceived to improve captive management, reproduction and welfare.
- EEP coordinators to collate research activities.
- Research advisor to report on activities and facilitate TAG wide research activities.

Section 1: Biology and field data

1.1 Taxonomy

1.1.1 Order

All rhinoceroses are placed in the order of Perissodactyla. Perissodactyla comes from the Greek word 'perissos', which means odd number and 'dactulos' meaning finger or toe in Greek (Huffman, 2007).

1.1.2 Family

The order Perissodactyla is comprised of three families; the Equidae (horses), the Tapiridae (Tapirs) and the Rhinocerotidae to which the rhinos belong (Nowak, 1999).

1.1.3 Genus

There are four genera of rhinos within the family. The Black rhino is placed in the genus *Diceros* (Nowak, 1999).

1.1.4 Species

The genus *Diceros* has one recent species, *Diceros bicornis*, the Black rhino which was first described by Gray in 1821 (Nowak, 1999). The name *Diceros bicornis* comes from Greek and Latin, *Diceros* from the Greek "di", meaning "two" and "ceros", meaning "horn" and *bicornis* from the Latin "bi", meaning "two" and "cornis", meaning "horn" (IRF, 2008).

1.1.5 Subspecies

There are four subspecies recognised within the Black rhino*;* the eastern ssp. *(*D.b. micheali*),* the south-western ssp. *(*D.b. bicornis*),* the south-central ssp*. (*D.b. minor*)* and the western ssp. *(*D.b. longipes*),* (Emslie, 1999) which has recently been reported extinct (IUCN 2011).

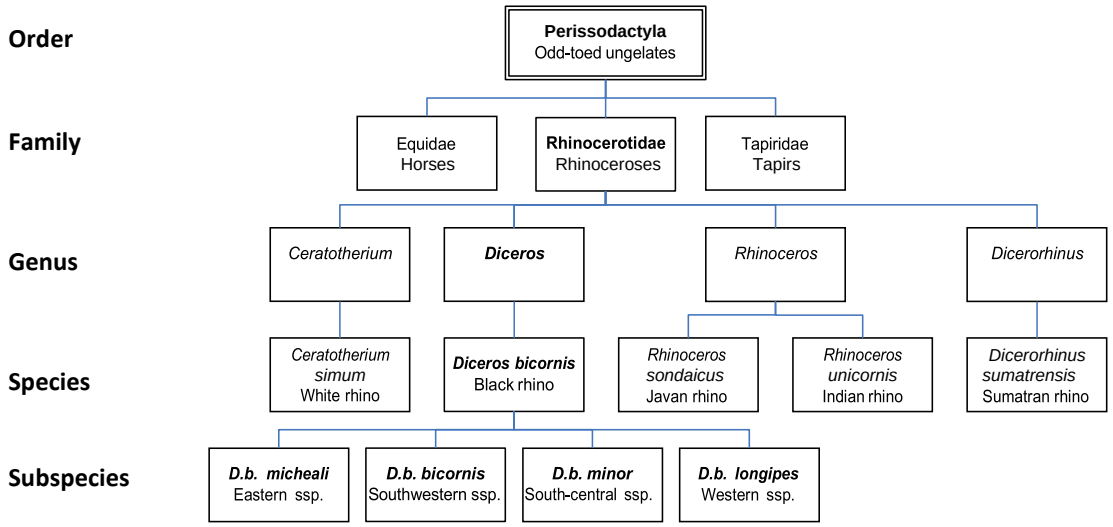


Figure 1.1. Classification of the Black rhino (*Diceros bicornis*) (Emslie, 1999; Nowak, 1999).

1.1.6 Common names

Black rhinos are actually not black at all. The name Black rhinoceros probably derives as a distinction from the White rhino (itself a misnomer), both species are grey. The White rhino having apparently derived its name from a variation of the early Cape Dutch word 'wijdt' meaning wide referring to the wide mouth of the White or Square-lipped rhino. Black rhinoceros can also refer to the dark-coloured local soil that often covers its skin after wallowing in the mud. Another common name for the Black rhino is prehensile or hook-lipped rhinoceros referring to the upper lip of the Black rhino which is adapted for feeding from trees and shrubs and it is its best distinguishing characteristic (Emslie, 1999; IRF, 2008).

Table 1.1: Translation of Black rhino into several European languages (Dollinger, 2008).

Languages	*Diceros bicornis*
Dutch	Puntlipneushoorn, zwarte neushoorn
English	Prehensile or hook-lipped rhino(ceros), Black rhino(ceros)
German	Spitzmaulnashorn
French	Rhinoceros noir
Spanish	Rinoceronte negro

1.2 Morphology

1.2.1 Body size

Adult Black rhinos have a body length of around 300 – 375 cm, and a height to the shoulder of approximately 80 – 140 cm. The weight of an adult Black rhino ranges between 800 and 1400 kg. Adult males are usually larger than females. The anterior horn is larger than the posterior horn; averaging about 50 cm in length. Sometimes the beginning of a third horn is present. The horns also differ between the sexes, with males tending to have chunkier horns and the females often longer and thinner ones. The longest recorded horn is 135.9 cm (Nowak, 1999).

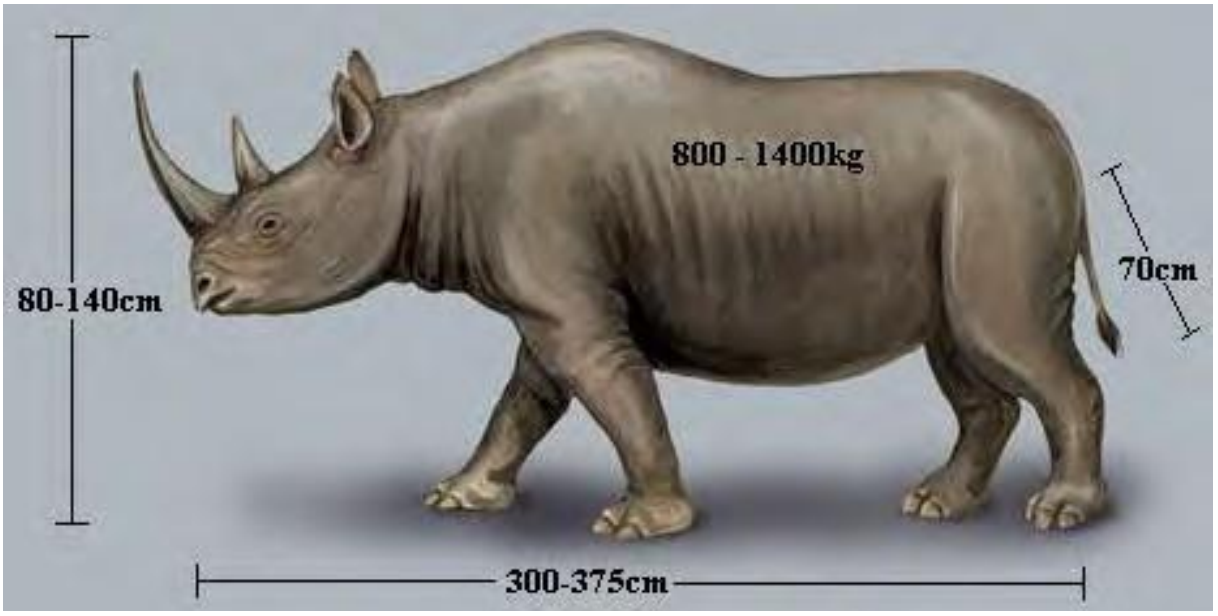


Figure 1.2 Body measurements Black rhino (Myers, 2006).

1.2.2 General description

The Black rhino's skin colour varies between pale grey to dark brown to dark grey and is greatly influenced by the colour of the local mud in which it wallows. An external feature which more clearly distinguishes the Black rhino from the white rhino is the protruding prehensile upper lip (Nowak, 1999).

The dental formula of the Black rhino is; incisors: 0/0, canines: 0/0, pre-molars: 3/3 and molars 3/3 with a total of 24 teeth (Nowak, 1999). Figures 1.3 to 1.5 show the dentition of a Black rhino.

Figure 1.3: Black rhino skull taken from the right.

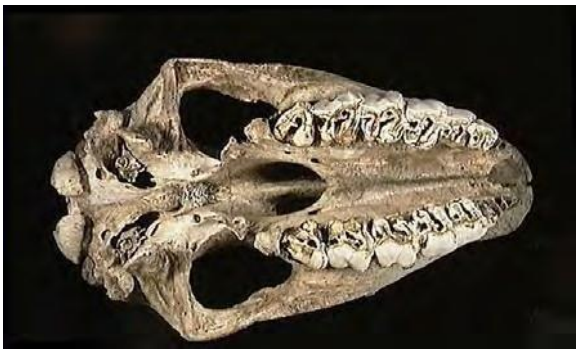


Figure 1.4: Upper jaw of a Black rhino.

Figure 1.5: Black rhino upper jaw molars.

Black rhinos have three toes with three stout nails, which leave impressions on the ground to the front and side of a softer wrinkled sole. The front feet are larger than the back feet (Adcock and Amin, 2006).

Black rhinos have two horns, which grow continually from the skin at their base throughout their life. The horn is continually worn away by rubbing. Each rhino develops its own rubbing habits and horn-wear patterns. Rhinos from different areas can have horns of different shapes (Adcock and Amin, 2006).

A Black rhino's sense of hearing is excellent, as is their sense of smell. These compensate for their poor eyesight which cannot easily detect an observer standing more than 30 m away. They can however detect movement at short distances (Adcock and Amin, 2006).

1.3 Physiology

The normal body temperature of a Black rhino ranges from 34.5 °C to 37.5 °C. The pulse is 30 to 40 beats per minute, and respirations are six to twelve breaths per minute (Fowler and Miller, 2003).

1.3.1 Horn

A rhino horn is comprised of thousands of compressed hair-like strands. The main component of the horn is keratin, making it extremely hard and tough, but it can be broken or split during fighting (Adcock and Amin, 2006).

1.3.2 Digestive system

The anatomy and digestive system in rhino species roughly resembles that of horses. Rhinos are monogastric animals with a hindgut-fermentation chamber. Microbial fermentation of plant fibre in the hindgut (cecum and large intestine) provides the main energy source for rhinos (Claus and Hatt, 2006). Figure 1.6 is a drawing of a rhino's digestive system.

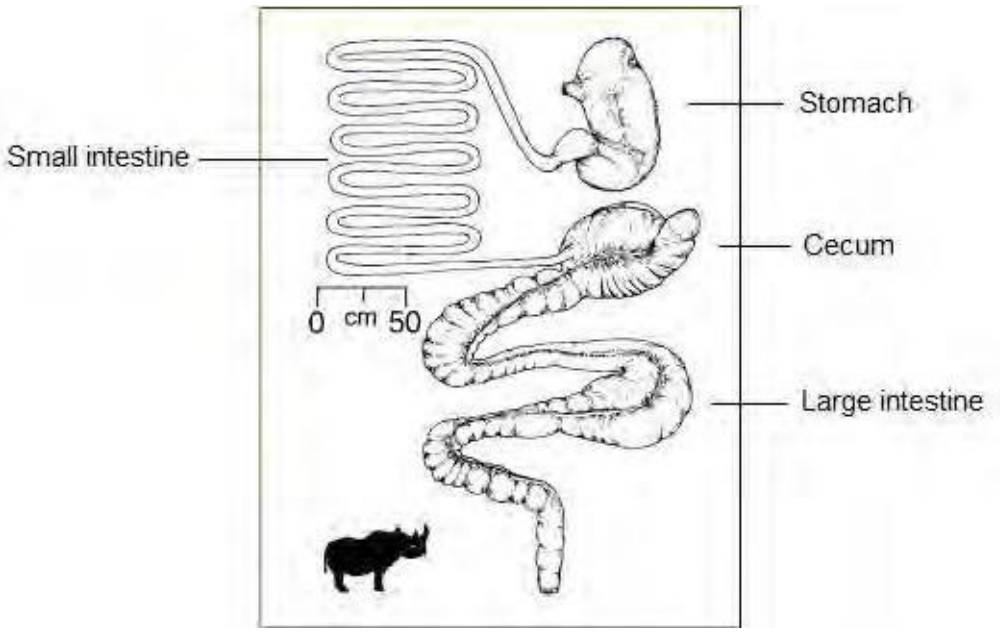


Figure 1.6: Black rhino digestive system (Stevenson and Hume, 1995).

1.3.3 Reproductive physiology – female

Female rhinos that have not bred have a hymen, the membrane that covers the vaginal opening which is often present cranial to the urethral opening. In several cases, a persistent hymen has been associated with a failure to breed, evidenced by the male's failure to achieve intromission after mounting. Rhinoceros have a long vagina characterised by longitudinal folds. These folds can make the opening of the cervix difficult to locate. The cervical canal is long and characterised by interdigitated folds. The uterus is bicornate, forming two horns after a short uterine body. The uterusof a pregnant rhino is characterised by diffuse placentation, meaning that almost the entire surface of the ventral outgrowth of the hindgut of the early embryo and the outermost membrane of the sac enclosing the foetus are used to form the placenta. The paired mammary glands are inguinal in position (Fowler and Millar, 2003).

1.3.4 Reproductive physiology – male

In the male Black rhino, the testicles are held close to the body along the preputial fold and are positioned horizontally as in the horse. The male reproductive tract includes vesicular glands, bulbourethral glands, and prostate. The relaxed penis is curved caudally, a position that results in the characteristic backward directed urination in male rhinos. The penis has notable horizontal flaps. Natural intromission may last up to 45 minutes (Fowler and Millar, 2003).

1.4 Longevity

In the wild Black rhinos can reach an age of 40 years. Black rhinos have the highest incidence among mammals of fatal interspecies fighting: almost 50 % of males and 33 % of females die from wounds. Fights between Black rhinos are usually for establishment and control of their territories. Why they are quite so aggressive is not known: in any event, rhino populations with high mortality rates recover only slowly. In captivity a male Black rhino has reached the age of 49 years (Felts, 2007; MacDonald, 2004; Nowak, 1999).

Field data

1.5 Zoogeography and ecology

1.5.1 Distribution

The Black rhino originally occurred throughout eastern and southern Africa and in the north ranged as far as north-eastern Sudan and at least as far west as north-eastern Nigeria (Figure 1.7). The extent of the former range in western Africa is not precisely known; suggestions are that prior to 1900 *Diceros* was found in the savannah zone as far west as Guinea (Nowak, 1999).

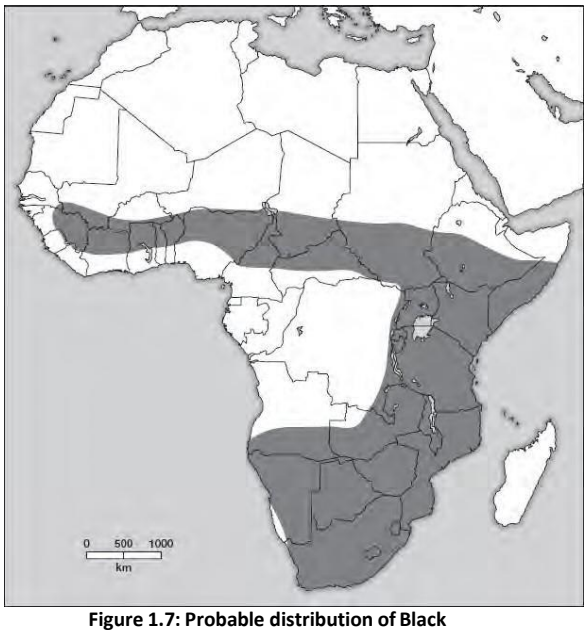


Figure 1.7: Probable distribution of Black rhino, circa 1700 (Emslie, 1999).

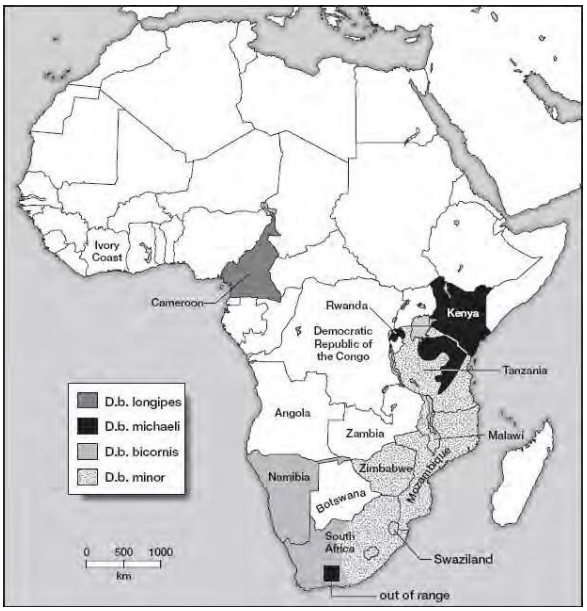


Figure 1.8: Distribution of the four Black rhino subspecies in 1997 (Emslie, 1999).

The Black rhino is divided into three extant and one recently extinct subspecies. The eastern ssp. (*D.b. micheali*) had a historical distribution from southern Sudan, Ethiopia, Somalia, and Kenya into northern-central Tanzania. The south-western ssp. (*D.b. bicornis*) had a historical distribution from Namibia, southern Angola, western Botswana and south western Africa. The original range for the south-central ssp. (*D.b. minor*) includes western and southern Tanzania, Zambia, Zimbabwe, Mozambique, northern and eastern parts of South Africa. It also probably occurred in southern Democratic Republic of the Congo, northern Angola and eastern Botswana. The western ssp. (*D.b. longipes*) recently reported to be extinct once ranged through west-central Africa *(Emslie, 1999).*

1.5.2 Habitat

The Black rhino is found mostly in the transitional zone between grassland and forest, generally in thick thorn bush or acacia scrub but also in more open country. It is not primarily a grassland animal but it favours the edges of thickets and extensive areas of short woody growth. Black rhinos exist wherever enough herbs and woody browse occurs in such sufficient amounts to support it. This

spans a wide range of habitats covering deserts, semi-deserts, wooded savannahs, woodlands, forests and even sub-alpine heathlands. Black rhinos are restricted to habitat within about 25 km of permanent water (Nowak, 1999; Amin, 2006).

1.5.3 Population and conservation status

Early in the 19th century the Black rhino was the most numerous of all the rhino species with a total number in the hundreds of thousands. Due to poaching for their horn, numbers crashed to a low of 2,410 in 1995. Since then Black rhino numbers have been slowly increasing however well equipped, well organised crime syndicates have killed more than 2,050 African rhinos since 2010 (May 2013, taken from AfRSG, Traffic and CITES Rhino Working Group). South Africa alone lost 668 rhinos in 2012 (more than double the number in 2010) and trends suggest this number may be exceeded in 2013 (367 by May 2013). Minimum numbers of poached African Rhinos show a drastic increase in recent years, rising from 60 in 2006 to 745 in 2012. Most rhino horns leaving Africa are destined forSoutheast Asian medicinal markets that are believed to be driving the poaching epidemic. In particular, Vietnamese nationals have been repeatedly implicated in rhino crimes in South Africa.

Despite these poaching losses, Black rhino numbers are up to 5,055 as of 31 Dec 2012 (from 4,240 in 2007). Even though this population growth is encouraging, unless the rapid escalation in poaching in recent years can be halted, African rhino numbers could once again start to decline. If the rate of increase in poaching seen 2011 / 2012 continues, modeling indicates that the tipping point when numbers start to decline could be reached as soon as 2015 (Emslie, IUCN / SSC AfRSG 2012).

In the year 2012 there were 799 eastern Black rhinos, 2,299 south-central Black rhinos and 1,957 south-western Black rhinos in the wild. The western Black rhino is extinct.

- The eastern Black rhino (*D.b. micheali*) is Critically Endangered, with a current stronghold in Kenya, with 631 rhinos as at the end of 2012. They live mostly within protected areas, (sanctuaries in both protected areas and on private land) and in free-ranging populations on county council land. Tanzania has *c. 100* eastern Black rhinos, mostly in free-ranging populations in unfenced protected areas and a few in sanctuaries. Rwanda and Ethiopia hold relict populations of one and two to four animals, in a protected area and on community land, respectively. At the end of 2012 South Africa had *c.* 68 eastern Black rhinos of predominantly Kenyan origin maintained on private land.

- Also categorised as Critically Endangered, the stronghold of the south-central Black rhino (*D.b. minor*) is South Africa and to a lesser extent Zimbabwe, with smaller numbers remaining in southern Tanzania. The south-central Black rhino is now thought to be extinct in Angola and Mozambique but small numbers have been reintroduced into Swaziland, Malawi and, more recently, Zambia and Botswana.

- Significant populations of the vulnerable south-western Black rhino (*D.b. bicornis*) have remained in the desert and arid savannah areas of Namibia and this country is the stronghold for the taxon, conserving 1,750 rhinos as at the end of 2012 with South Africa conserving a further 206 rhinos. There are no south-western Black rhinos in captivity.

- The population of the western Black rhino (*D.b. longipes*) was reduced to only a few scattered animals remaining in northern Cameroon with some animals believed to be seasonal visitors to Chad. The last extensive survey of possible rhino range in 2006 failed to find any rhino or signs of rhino, and there have been no sightings since 2006. This subspecies is now categorised as extinct (Amin, 2006; Emslie, 2006; IUCN, 2011).

1.5.4 Threats

The Black rhino faces a variety of threats. One of the main threats is poaching for the international rhino horn trade. Rhino horn has two main uses; use in traditional Chinese medicine, and ornamental use (for example, rhino horn is a highly prized material for making ornately carved handles for ceremonial daggers (Jambiyas) worn in some Middle East countries). During the 1960s civil unrest and the free flow of weapons in Africa had a significant impact on African rhino conservation efforts. Black rhino populations in Angola, Central African Republic, Chad, Mozambique, Namibia, Rwanda, Somalia, Sudan and Uganda have to varying degrees all suffered from the consequences of war and civil unrest since the 1960s. Some detrimental effects include trading of rhino horn and ivory for weapons, increased poaching due to increased poverty in times of civil unrest, and diminished levels of protection for rhino populations as funds are diverted away from wildlife departments. Habitat changes can also cause rhino populations to decline (African rhino specialist group, 2003).

Rhino horn has been an integral component of traditional Chinese medicine for thousands of years. It is ground down, mixed with water and ingested and believed to to be effective in reducing temperature, treating high fevers and convulsions, controlling haemorrhaging and assisting the liver in cleansing the blood of toxins resulting from the intake of alcohol or poison. Trade patternsdetected by TRAFFIC indicate that the resurgent demand for rhino horn is driven primarily by users from Vietnam. Increasing prosperity in the Vietnamese economy has led to increased levels ofindividual disposable income and, sadly, use of rhino horn appears to be a way to demonstrate one's affluence and high social status (Traffic 2012).

Threats to eastern Black rhinoceros: Some populations of the eastern Black rhinoceros in enclosed areas appear to be overstocked and are showing clear signs of density-dependent reductions in reproductive performance. In some cases competition from other browsers, such as African elephants *Loxodonta africana* and Giraffes *Giraffa camelopardalis,* appears to also be negatively affecting rhinoceros carrying capacity. Limited budgets for conservation are also a problem.

Threats to south-central Black rhinoceros: Conservative biological management appears to have limited metapopulation growth rates in some key populations. In parts of Zimbabwe, land transformation following re-settlement has negatively affected habitat in some areas and has resulted in a number of snare-related deaths. There is a plan to create an additional intensive- protection zone in Zimbabwe. Declining conservation budgets, an apparent increase in poaching and losses of animals to snaring, and the prosecution of rhinoceros offences under statues withoutdeterrent sentences are of concern.

Threats to south-western Black rhinoceros: Illegal hunting has been blamed for the disappearance of the *s*outh-western Black rhinoceros from arid habitats in at least two range states (Angola and Botswana). Since 1979 conservation efforts in Namibia have stemmed poaching activities and the population has increased steadily. As in other range states, declining budgets for conservation are a problem.

Threats to Western Black rhinoceros: Poaching, lack of finance, limited anti-poaching efforts, limited local capacity for conservation management, failure of courts to give sentences that can act as a deterrent to potential poachers and genetic / demographic factors all pose serious threats to this subspecies.

1.5.5 Conservation actions

The Black rhino is, as a full species, classified as Critically Endangered by the IUCN. The south-central, eastern and western subspecies are also classified as Critically Endangered. The south-western subspecies is classified as vulnerable (IUCN 2012).

Black rhinos have been listed on CITES Appendix I since 1977. All international commercial trade in Black rhinos and their products have been prohibited. To help reduce illegal trade and complement CITES international trade bans, domestic anti-trade measures and legislation were implemented in the 1990s by a number of consumer states. Effective field protection of rhino populations has been critical. Many remaining rhinos are now concentrated in fenced sanctuaries, conservancies, rhino conservation areas and intensive protection zones where law enforcement effort can be concentrated at effective levels. Monitoring has also provided information to guide biological management decision-making aimed at managing rhino populations for rapid population growth. This has resulted in surplus animals being translocated to set up new populations both within and outside the species' former range. Following a decline in breeding performance in some areas, increased effort has recently been given to improving biological management with a view to increasing metapopulation growth rates. Increasing efforts are also being made to integrate local communities into conservation efforts. Strategically, Black rhinos are now managed by a range of different stakeholders (private sector and state) in a number of countries increasing their long term security. In contrast to southern White rhino, most Black rhino on privately owned land are managed on a custodianship basis for the state. In addition to local and national initiatives, there are a number of regional African rhino conservation initiatives: the South African Development Community (SADC) Regional Programme for Rhino Conservation, the SADC Rhino Management Group, the SADC Rhino Recovery Group, and the Southern African Rhino and Elephant Security Group. IUCN SSC's African Rhino Specialist Group is the continental coordinating body for rhino conservation in Africa (African rhino specialist group, 2003).

1.6 Diet and feeding behaviour

1.6.1 Food preference

The Black rhino is a browser, its main foods being the thin regenerating twigs of woody growth and legumes. A great variety of plant species are utilised, although acacia seems to be a favourite(Nowak, 1999). The natural diet of the Black rhinoceros is characterised by a high fibre and moderate to high protein content (Claus and Hatt, 2006). They eat a wide range of browse species in any given habitat, but while over 100 species may be ingested during a year's foraging, 90% of the diet is commonly made up from fewer than 20 species. Grass is generally only eaten incidentally while foraging for low-growing herbs, but new soft grass leaf growth is voluntarily taken (Adcock and Amin,2006).

1.6.2 Feeding

Twigs are gathered with the prehensile upper lip, drawn into the mouth, and snapped off with the premolars. Drinking occurs every day if water can be reached and mineral licks are visited regularly (Nowak, 1999). Black rhinos are most active during the night-time when most of their foraging and drinking is done. Foraging also occurs in the cooler hours of the morning and afternoon, but wallowing and / or sleeping in a cool, breezy or shady spot is the main activity during the heat of the day (Adcock and Amin, 2006).

1.7 Reproduction

The sex determination system of XX / XY is applicable on Black rhino, where the males have XY and females have XX chromosomes. Black rhinos have a total of 84 chromosomes (Trifonov, 2003).

1.7.1 Sexual maturity

In wild males sexual maturity is reached between seven to nine years and in wild females at between four to six years. The first parturition of females in the wild is estimated around five years, but in some populations it might take up to twelve years. Research found that in two South African subpopulations of Black rhino, in which there were 0.1 / km^2, that in these two subpopulations females reached sexual maturity at a younger age then in a third subpopulation with a Black rhino density of 0.7 / km^2 (Adcock and Amin, 2006; Carlstead et al, 1999; Nowak, 1999).

Records from captive Black rhinos in the EEP show the youngest male at first reproduction was three years old. However, more generally (the youngest ten males at first reproduction, out of the total past EEP dataset) range between four and five years old. The average age at first reproduction is ten years and one month.

The youngest female at first reproduction was five years old, the youngest ten females at first reproduction ranged between five and six years old. The average age for a female at first reproduction is ten years and four months.

1.7.2 Seasonality of cycling

The recurring of the cycle in regular intervals is more important than the length of the cycle in days. Oestrous cycling begins while the mother is still nursing. Generally, breeding occurs throughout the year; however there may be mating peaks in some areas. In Kenya mating peaks occur during September till November and during March till April. In Zululand mating peaks are during October till November and during April till July. These indications suggest that most births take place in the rainy season (EAZA yearbook, 1995; Hutchins, 2003; Nowak, 1999).

Irregular oestrous cyclicity patterns are relatively common among captive black rhinos (see section ***1.7.3*** *Reproductive cyclicity in females*), and in many females behavioural expression of oestrus can also be difficult to detect. It is a recommended that collections use hormone analysis as a management tool as this can allow the prediction of when a female will be receptive to the male based on her hormone profile, giving keepers extra information to help introduce the rhinos at the right time. Furthermore, once pregnancies have been confirmed, this allows the male to be separated and mixed with another female at an earlier stage of gestation.

As oestrous cycle length can be highly variable both between females and within an individual female over time, long-term sample collection is the best way to make accurate predictions. At Chester Zoo, since the initiation of the endocrine programme there have been six pregnancies and five births in six years, highlighting the impact this additional tool can have on facilitating breeding management.

1.7.3 Reproductive cyclicity in females

Black rhinos are polyoestrous, meaning that they will come into oestrus, i.e. the time of receptivity to the male, on multiple occasions throughout the year. Although there is some evidence of seasonality of births in the wild due to weather conditions (Hitchins and Anderson 1983), and perhaps in zoos due to management constraints, there is no evidence of seasonality in oestrous cycles.

The oestrous cycle has previously been characterised in this species using steroid hormone analyses, either using blood (Berkeley *et al.* 1997), urine (Hindle *et al.* 1992) or faeces (Brown *et al.* 2001; Edwards et al. 2013), and saliva could potentially also be used as a sampling medium. Changes in the ovary during the oestrous cycle have also been previously described using ultrasound (Radcliffe *et al.* 2001).

An average oestrous cycle length of the Black rhino has previously been described as around 26 days in length; however, research on wild (Garnier *et al* 2002), and captive rhinos in America (Brown *et al.* 2001), and more recently in Europe (Edwards *et al.* 2013) have revealed that oestrous cycles are more variable in length, with typical oestrous cycles lasting between 20 - 40 days. In addition, short (< 20 days), and long (> 40 days) cycles, and acyclic periods with no evidence of oestrous cyclicity are also relatively common. The causes of these different cycle types are yet to be fully understood, but

early indications suggest that these may not reflect normal reproductive function, and to-date pregnancies have only been reported associated with 20 - 40 day cycle types. Therefore oestrous cycles of 20 - 40 days in length are considered to be normal, and although females may vary in their typical cycle length, within an individual cycle length tends to be relatively consistent. Indeed, the regularity of oestrus on an approximately monthly basis is perhaps more important than the exact length of the oestrous cycle.

Females in captivity may commence oestrous cyclicity between three and four years of age, with one female age three years eight months of age exhibiting clear oestrous cyclicity (Edwards *et al.* 2013). The oldest female to have reproduced in captivity was aged 32 years, but females may continue to cycle after this time, and in the wild have been reported to continue producing offspring after 30 - 35 years.

1.7.4 Reproductive hormones in males

Testosterone can be measured in blood (Christensen *et al.* 2009) and faeces (Brown *et al.* 2001; Edwards *et al*. 2013), and can be used as an indicator of reproductive function. Testosterone concentration increases with age (Edwards *et al.* 2013), and may also vary according to prior reproductive success (Edwards et al. 2013), or due to the sociosexual environment (Christensen *et al.* 2009).

1.7.5 Gestation period / birth rate

The gestation period is around 15 to 16 months or 440 to 460 days. The inter-birth period in the wild is somewhere around 27 months. The inter-birth period in captivity is 40 months. This may be due to delayed reintroduction to a mate postpartum (Carlstead et al, 1999; EAZA yearbook, 1995; Hutchins, 2003).

1.7.6 Birth

After the gestation period there is a single calf born, which weighs around 40 kg. Birth usually takes place in the early morning (*Nowak, 1999*).

1.7.7 Development

Nursing generally continues for over one year, and the older calf is driven away by the mother around the time that the next offspring is due. Some solid food may be taken within a few weeks of birth, weaning is completed after about two years; independence is achieved between two and a half and three and a half years old (Hutchins, 2003; Nowak, 1999).

1.8 Behaviour

The Black rhino is unpredictable in its behaviour and can be a dangerous animal, sometimes charging a disturbing sound or smell. It has tossed people in the air with the front horn and regularly charges vehicles and campfires. If a Black rhino catches the scent of humans, it usually runs away, sometimes for quite a distance before stopping (Nowak, 1999).

1.8.1 Activity

Black rhinos are more active (feeding, drinking and walking) in early morning and late afternoon to evening. Black rhinos are also active at night, often feeding, drinking, and walking outside their core areas and in more open habitat than during the day. The most intensive feeding takes place during early morning and evening.

Sleeping occurs either standing or lying down. Black rhino react swiftly when disturbed from rest, usually standing up and facing the source of disturbance. Because they have poor eyesight they may not locate the disturbance easily. Being curious animals, they will walk or trot forward to find out what is going on.

Black rhino usually run away if they catch a human's scent and only charge if they feel threatened. Black rhinos frequently wallow in shallow water holes. The water helps them to keep cool, they coat themselves in mud, probably to gain a protective coating against biting insects.

The horn is continually worn away by rubbing. Each rhino develops its own rubbing habits and horn-wear patterns. Rhinos from different areas can have horns with different shapes (Adcock and Amin, 2006; Jansa, 1999; Massicot, 2007; Nowak, 1999).

1.8.2 Locomotion

Black rhino can move extremely fast. They can run at 55 kilometres per hour change direction surprisingly quickly. They can run right through scrub and bushes (Adcock and Admin, 2006; Nowak, 1999).

1.8.3 Predation

Adult Black rhinos have no predators, although lions, leopards and hyenas may kill calves and sub-adults. Evidence of predator attacks are sometimes seen in the form of mutilated ears or missing tails. According to Brain, Forge and Erb (1999) sub-adult Black rhinos of a certain age appear particularly susceptible to lion predation in Etosha National Park. The sub-adults at this age have just left their mothers and are still relatively small. Brian, Forge and Erb (1999) also report that over a 13 year period in the Hluhlwe / Corridor / Umfolozi game reserve complex, there were no records oflion predation on Black rhinos, although there was strong evidence to suggest that there was spotted hyena (*Crocuta crocuta*) predation on small calves. It is estimated to be a 16 % loss of rhinos less thantwo years old to predation. Black rhinos are also capable of killing their predators. Reports vary from

females with calves killing lions and a sub-adult female killing an adult hyena (Adcock and Amin, 2006; Brian, Forge and Erb, 1999; Law and Myers, 2004).

1.8.4 Social behaviour

Group structure: Black rhinos are predominantly solitary, the most commonly observed group structures being adult females with young. Other groups of various ages and genders occur, but they are usually temporary. The largest temporary group reported in one study included 13 Black rhinos. Recent studies indicate that Black rhinos are more social than previously thought and particularly around waterholes at night. Females are usually are found together with a calf and sometimes an older daughter. Females without young may temporarily join a neighbouring female. Sub-adults frequently associate with other Black rhinos. Only fully adult males become solitary, and even then they may form temporary groups that move and feed together. Male Black rhinos only become socially mature when they establish a set territory, in which they spend most of their time and do most of their feeding. Females settle into their own home range near the time of birth of their first calf. Female home ranges can overlap. Dominant bulls do not overlap with their home ranges (Adcock and Amin, 2006).

Relations and communication: Adult male Black rhino tend to live on their own, except whencourting females. Among males, there are dominant and subordinate animals. Subordinate rhino are often sub-adults who must defer to an established territorial bull or risk a fight. Young bulls are often killed or injured in these interactions. Old males which can no longer defend their territories also die in fights, or become confined to a small area (Adcock and Amin, 2006; Massicot, 2007).

Black rhinos that share a part or all of their home range exhibit a familiarity with one another instead of the aggression that they exhibit to total strangers. Black rhino advertise their presence in their range to other rhino by spray-urinating and scraping their dung on the ground next to a path; and also by defecation on well-developed dung-piles. Male rhino spray-urinate and scrape more than females, and territorial (dominant) males keep more dung-piles in and around their range (Adcock and Amin, 2006; Nowak, 1999).

The explosive puffing snort of an alarmed Black rhino is the sound most clearly associated with this species by people who work with them. An appealing high-pitched whine or squeal is another sound made by this species. The high-pitched whine is used by calves to attract its mother's attention, a male may use it to court a female, and all Black rhinos use it when in pain or in distress (Adcock and Amin, 2006).

1.8.5 Sexual behaviour

An adult male and female, with the female's young if she has one, form temporary associations for mating during the female's oestrus. For a few days, when the female is in oestrus, a pre-mating bond may develop between the bull and the cow, and the pair remains together during resting and feeding. They even sleep in contact with each other. Young are sometimes attacked by males during courtship. The young return to the female when the oestrus is over. Although at times several bulls may court a female simultaneously without apparent antagonism, serious fights and frequent deaths

result from conflicts over females in oestrus. Rhinos are renowned for the extended duration of copulations, which last between 20 minutes and an hour or longer, with multiple ejaculations (Adcock and Amin, 2006; Hutchins, 2003; Massicot, 2007; Nowak, 1999).

Section 2: Zoo management

Introduction

This section suggests best practice management of Black rhino in the zoo environment. This topic is divided into the following chapters: Enclosure, Feeding, Social structure, Breeding, Behavioural Enrichment, Handling and Veterinary. The information for this has come from three main sources. Firstly, the opinions of experienced Black rhino managers were sought via a questionnaire and their opinions integrated into the first draft guidelines. These were: Andreas Knieriem (Hannover Zoo) Gerd Nötzold (Leipzig Zoo) Jiri Hruby (Dvur Kralove Zoo) Robert Zingg (Zurich Zoo) Ulrike Cyrus (Zurich Zoo) Helen Massey (NEZS Chester Zoo) and Xavier Vailliant (Pont-Scorff Zoo). Secondly, information was shared and compiled as a result of the discussions at a Black rhino husbandry workshop held in Doué le Fontaine, France in May 2010. Thirdly, information has been included from the AZA Rhinoceros Husbandry Resource Manual (Fouraker, M. and Wagener, T. 1996) additionally some other relevant sources of information have been used and these are referenced in the text.

A review of these Best Practice Guidelines was completed in 2013 by Becca Biddle and Dr Mark Pilgrim. For this we are especially grateful to Dr Andreas Ochs of Berlin Zoo for his review of the Veterinary Section, Marcus Clauss and Jürgen Hummel for their work on the Nutrition Section, and DrSue Walker and Dr Katie Edwards for their additions regarding Black rhino endocrinology and its applications to captive management. Many thanks to all involved.

2 Enclosure

In the temperate European climate Black rhino require indoor as well as outdoor facilities and each of these facilities is described separately. When designing an enclosure it is important that the zoo environment resembles the natural environment as closely as possible to maximise rhino health and reproductive success, when talking about indoor enclosures this may refer to temperature andlighting.

It is recommended that new holders plan for 2.3 rhinos. However if necessary this may be phased, with an initial build for a minimum of 1.2 rhinos and a commitment to be able to house 2.3 within five years.

2.1 Indoor enclosure

2.1.1 Indoor boundary

The recommendation is to build for 2.3 animals, with a minimum of six indoor enclosures. If the building is being phased the initial build must be for at least 1.2 animals with a minimum of four indoor enclosures. These indoor enclosures should be adjacent to and interconnected with each other allowing flexibility to combine enclosures to create fewer larger areas or split them to provide individual areas for each of the three adults and potential calves / juvenile. Adjacent enclosures

should be accessible through at least two gates, giving the animals the possibility to roam without danger of being trapped (EAZA yearbook, 1995).

At least one of these areas should be suitable for the isolation of an animal (e.g. new animals or sick animals). This area should be off show to zoo visitors, protected from disturbance (such as noise) and should not allow direct contact between animals.

The boundary between the indoor enclosures separating the rhinos from each other should generally be solid walls with an area in the boundary to allow visual and limited physical contact between the animals. These have proved very useful in getting individuals used to each other prior to mixing and to allow keepers to better evaluate when animals are in oestrus using behavioural cues. The isolationpen should be of all solid walls and not allow contact between the animals.

The boundary between the rhinos and the keepers may be solid or bars. Bars have the advantage of giving the keepers an opportunity to habituate the animals to be touched all over their bodies. This facilitates health examinations and veterinary treatments, provides good opportunities for operant conditioning (training) and also promotes a good bond between keepers and rhinos (Figure 2).

Facilities should be designed so that each rhino can be checked over or trained on a daily basis should this be required. It is preferable that these training or examination areas are inside or undercover, however some collections do make use of outdoor training areas (either in addition or instead of indoor areas) to good effect (see *sections 2.7 and 2.8 on Handling and Veterinary 2.7*).

Figure 2: Indoor barrier between rhinos and keepers that allows keepers close contact to the rhinos. Note the low horizontal barrier that prevents a young calf passing through the bars.

Walls are usually made of concrete, concrete clad with wood, or wood of a suitable strength. Wood cladding on the concrete walls may help to prevent the rhinos from excessively rubbing their horns.

Bars are usually vertical, however horizontal and diagonal steel may also be used with great care taken to ensure that the bars are far enough apart to reduce the chance of rhinos trapping theirhorns and damaging them. Additional barriers may need to be required at low level when housing young calves to prevent them passing through. Vertical bars should be spaced 25 - 30 cm apart (Fouraker and Wagener, 1996).

Enclosure gates will often be the weakest points of the exhibit and therefore adequate hinge and lock strengths are very important. Interior doors are usually constructed of heavy-gauge galvanized steel or pipe that is hinged or sliding. Manual sliding gates are preferred to swing gates or hydraulic gates both for their speed of closure and to reduce the chance of keepers getting trapped or injured. Gates should be constructed to allow keepers to open and close them without entering rhino space.It is also important that keepers have good visibility either side of the gates in order to operate them safely. When sliding gates are used, the track must be kept clean in order to reduce the chance of them seizing, and care should be taken in the construction of the track to avoid injuring the feet of the animals as they run through gates during introductions. All gates must be firmly secured and designed so the animals cannot dislodge them with their horns, i.e. constructed in a way that the gate cannot be lifted off its hinges by a rhino.

2.1.2. Indoor substrate

Brushed concrete is most commonly used as the indoor substrate however tiles and rubber coated concrete may also be used. It is recommended that animals have access to both hard and softer substrates in their enclosure; these could be either inside or outside. If sand or other loose substrates are used, avoid feeding on these as this may promote accidental ingestion of the substrate and subsequent digestive problems.

2.1.3. Indoor furnishing and maintenance

Some collections provide the animals with straw bedding (Figure 2.1). Rhino urine can be sticky and corrosive and therefore enclosure furniture should be able to be scrubbed clean. No delicate equipment should be stored within spraying distance of the rhinos.

Figure 2.1: Straw bedding on a brushed concrete floor.

Indoor enclosures should be scrubbed clean with water on a daily basis. Cleaning products such as disinfectants with a strong smell should be avoided for routine cleaning as these are disliked by rhinos (Carlstead et al 1999).

Wooden rubbing posts should also be available. Horizontal pieces of wood have proved popular in one institution as they allow the rhinos to mimic the behaviour seen in wild rhinos where animals rubboth the inside and outside curvatures of the horn. Availability of these horizontal wooden bars seems to promote more similar shaping of the horn to that seen in the wild.

2.1.4. Indoor environment

An indoor temperature of 18 - 20°C should be maintained. Indoor temperatures of greater than 30°C should be avoided. Various heating methods have been used successfully including radiators, hot water pipes, radiant panel heaters, hot air blowers and under-floor heating. If under-floor heating is used it is advisable for it to be installed in the area of the enclosure most likely to be used for sleeping, but not throughout the whole facility. This provides the animals with a thermal gradient and gives them a degree of choice as to where they are most comfortable sleeping. Some institutions have reported increased dust and ammonia fumes in houses where under-floor heating is used, so increased ventilation may be required to compensate. Indoor facilities should be maintained with good ventilation while avoiding draughts (EAZA yearbook, 1995).

Some institutions have used heavy duty plastic hanging strips on the doorways allowing animals free access to both indoors and outdoors whilst minimising heat loss and draughts. Animals can be habituated to these by installing one strip at a time. Most animals accept them within two - three weeks.

Thermal imaging cameras can be a useful tool in assessing surface temperatures in different parts of the indoor enclosure and identifying and eliminating cold spots. This is particularly useful when preparing an enclosure for a winter calving. One institution has experienced hypothermia in a newborn calf due to it lying on a cold floor. Use of deep straw (30 - 40cm) to cover the floor around the time of birth and during calf rearing is another method of reducing cold spots and recommended by one highly successful institution.

Natural daylight cycles seem to be adequate for rhinos. However, if an animal is to be held indoors for more than twelve hours (e.g. during winter in cold-climate institutions), facilities should provide artificial or natural light sources to stimulate natural cycles. Skylights or windows providing natural light should be included whenever possible.

Water should be available in each indoor area. Both standing water and wall mounted self-filling water troughs have been used. In order to avoid drowning when young calves are present, any water troughs set into the floor should either be replaced with one off the ground or drained of all but a few centimeters of water.

2.1.5. Indoor dimensions

It is recommended that in northern and central Europe where rhinos are likely to spend a great deal of time indoors, indoor enclosures should be no less than 60 m^2. In southern climates and if the animals have access to outdoor areas, the indoor pens can be smaller, but never less than 30 m^2.

2.2. Outdoor enclosure

2.2.1 Outdoor boundary

At least five outside areas are required for 2.3 animals, or three outside areas for 1.2 animals. These areas should be adjacent to and interconnected with each other allowing flexibility to combine enclosures to create fewer larger areas. The area used for mixing rhinos should offer multiple escape routes for the animals and good observation points for the keepers so that the animals can be separated quickly and safely if required.

A variety of methods have been used as primary barriers, including horizontal metal rails (Figure 2.2), vertical posts of either metal or wood and solid walls of concrete, masonry or stone (Figure 2.3). Black rhinos may climb and a primary barrier should be a minimum of 1.5m (5ft) high and non- climbable. There have been a couple of reports of particularly adventurous individuals getting just their front legs over horizontal rail fences of this height. Care should also be taken that animals cannot move items of cage furniture (such as logs) up to the fence line and use these as steps to climb over.

Posts connected by horizontal heavy gauge wire (hawser wire) have also been used however care should be taken with these as some rhinos will use them to rub their horns leading to the development of deep groove or even amputation of the horn. This behaviour may be reduced if plenty of alternative horn rubbing opportunities are available.

Figure 2.2: An example of a horizontal metal rail barrier.

Figure 2.3: A 2.0 meter high concrete barrier on a gently sloping ditch.

Both dry and water filled moats have also been used. Dry moats have a preference above water filled moats because of danger of drowning, especially if young calves are kept in the enclosure. Where dry moats are used, steep drops should be avoided as there have been cases of animals falling or being pushed into them. Ditches with vertical walls are considered dangerous and are not recommended, especially not in areas where animals may be introduced to other rhinos (Figure 2.4). The recommendation is that where existing vertical walls have ditches then these ditches be modified to a gradual slope with exits on either side of the ditch being provided (EAZA yearbook, 1995).

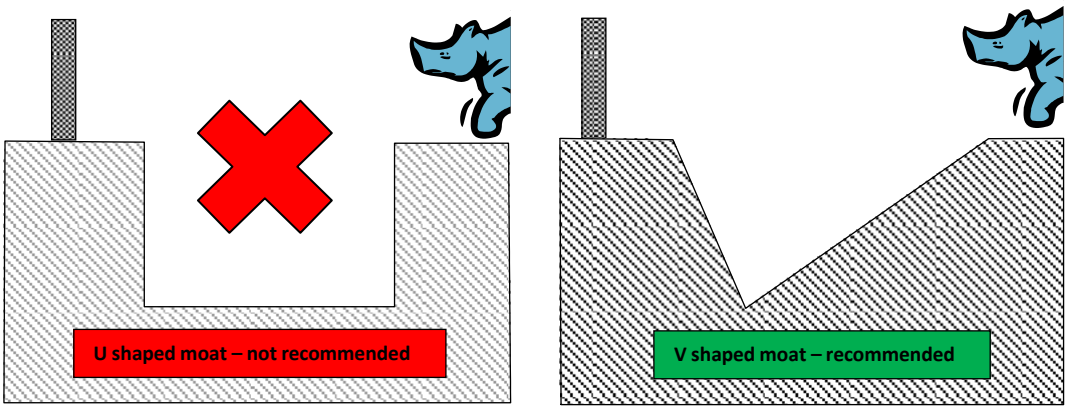


Figure 2.4: U shaped moat is not recommended, V shaped moat is fine.

Any moat used should have sloped areas to allow animals to exit easily if they enter them. It may be necessary to drain or otherwise prevent access to water moats or other areas of deep water in winter as there is a danger of animal attempting to walk on ice and slip or fall through.

It may be useful to have an area of bars (preferably vertical ones) where the keepers can examine and train the animals outside as well as inside as this will facilitate care of the animals without the need to bring them inside. Vertical pipes or posts should be spaced 25 cm to 30 cm apart (Fouraker and Wagener, 1996). Open bars and gates between the rhino paddocks are also important to allow socialisation of the animals prior to mixing. It is recommended that all introductions of animals be undertaken outside. Note, if bars are used, care should be taken to ensure that they are far enough apart to reduce the chance of rhinos trapping their horns and damaging them. If using poles, each should be about 30 cm thick and set into the ground in concrete. Bars or poles should be connected vertically to prevent the fence from being uprooted by the animals. If calves are kept outdoors, adequate measures are to be taken to prevent escaping. It is important to consider fence spacing and keeper access respectively in order to provide an exit in cases of emergency. When poles are used they must be treated with non-toxic compounds only.

Secondary barriers used to protect planting or provide escape areas for other species sharing the enclosure include fallen logs, large rocks (Figure 2.9) and lengths of primary barrier fencing. Electric fencing has been used with mixed results. Some animals respect the fence after their first shock; others seem to be irritated by it and will just rip it out. It certainly would not be a sufficient deterrent to stop a frightened or excited animal. Aprons of small sharp rocks have been tried to discourage rhinos from walking on particular areas however these do not seem to have worked and are not recommended. Triangular profile metal strips set over a shallow pit (cattle grid) have been used in one institution to restrict access to part of a paddock. This would almost certainly not deter an excited or frightened rhino and are also not recommended. To prevent individuals from being hurt, barriers should have no sharp edges (EAZA yearbook, 1995).

A variety of gate systems have been used. As with the gates used in the indoor enclosures, in general manual slides are preferred outside to swing gates or hydraulic gates both for their speed of closure and to reduce the chance of rhinos getting trapped or injured. It is important that keepers have good visibility of as much of the paddock as possible either side of the gates in order to operate them

safely. When sliding gates are used, the track must be kept clean in order to reduce the chance of them seizing. All gates must be firmly secured and designed such that the animals cannot dislodge them with their horns (e.g. cap hinges so that rhinos cannot lift gates off their hinges with their horns).

It is important that animals are adequately protected from excessive disturbance from visitors. Carlstead (1999) showed that mortality since 1973 correlated positively with percentage of public access. It is difficult to make recommendations as to a suitable maximum percentage of the perimeter to which visitors be allowed access as this will depend on topography and size of the enclosure, availability of visual and auditory barriers, number and behaviour of the visitors and the temperament of the individual animals. However as a general rule it would be inadvisable to allow public to access the whole perimeter and it is important to monitor the animal's behaviour closely and to be able to make adjustment as necessary. Public should be prevented from touching or feeding rhinos, suitable standoff fencing is probably the most effective way of achieving this.

2.2.2 Outdoor substrate

Grass, soil, sand, concrete and stone have all been used successfully. It is recommended that animals have access to both hard and softer substrates in the range of enclosures to which they have access. If sand or other loose substrates are used, avoid feeding directly on these as this may promote accidental ingestion of the substrate and subsequent digestive problems. If grass or soil is used it should be taken into account that this surface may not be suitable when very wet or cold and an outside area with a suitable all weather surface should be available. Sand or concrete aprons around houses, the fence line, gateways or other high use areas are recommended to prevent these areas becoming eroded and excessively muddy. When using an area for mixing of animals, make sure that the ground conditions provide a good footing and avoid waterlogged or very slippery surfaces.

Figure 2.5: A large outdoor paddock with a sand substrate at Zoo de Doue la Fontaine, France.

2.2.3 Outdoor furnishing and maintenance

The way an enclosure is furnished can make a big difference to its suitability for mixing rhinos. Visual breaks either using the natural topography of the paddock or by planting or building artificial barriers is important to allow animals to get out of view of other animals and visitors.

A fresh water source should be constantly accessible (EAZA yearbook, 1995). Water should be changed daily. Drinking water should be offered in a water trough which should be concrete, automatic-fill or a continuous-flow device. Regular cleaning should occur at a rate that inhibits the growth of algae and bacteria. Water devices should be substantially constructed to prevent injury, upset, spillage or leakage (Fouraker and Wagener, 1996) and preferably be placed in the corner of the enclosure. It is important that these water containers have no sharp edges or corners that could injure a rhino.

Black rhinos need and enjoy access to pools and or mud wallows for skin health, temperature regulation and behavioural enrichment (Figure 2.6). The size of mud wallows should be gauged by the number of animals in the exhibit to allow ample room for each individual. Rhinos will construct their own mud wallow when given a helping start by digging out a wet area of the paddock. Mud wallows should be renovated periodically to prevent contamination.

Figure 2.6: A young Black rhino keeping cool in a mud pool.

Pools are occasionally used by Black rhino in very warm conditions, however generally they prefer mud wallows rather than water pools. Any pools used should be shallow, between 0.3 m to 1.0 m deep. To allow the rhino to safely access the pool, ramps are preferable to steps and should have a slope not greater than 20^{o}. Ideally ramps in and out of the pool should be in place around the entire perimeter, or at least in two locations around the pool. Multiple entry sites into a pool prevent it from being a dead-end in the enclosure. In the design of slopes or steps, keeper access for cleaning should be considered. The pool substrate should be broom-swept concrete to prevent it from being too slippery. Pools should be located in areas that are shaded for at least part of the day (Fouraker and Wagener, 1996). Rubbing posts may be particularly effective if placed near mud wallows or pools. Post material must be non-toxic to rhinos.

Free access to shade is essential, so sun shelters or shade in the form of trees or other vegetation must be provided. It is recommended that a number of adequate shady zones are provided, which can be natural or built structures. Sun shelters should also be usable as rain shelters; trees rarely serve this purpose (Figure 2.7).

Figure 2.7: An outdoor rain / sun shelter

Indoor enclosures are not acceptable for sun protection unless they are accessible at all times. In parts of the enclosure protection from the wind should be provided (EAZA yearbook, 1995).

Any new exhibit should include the capability for video recording systems indoor and outdoor. In addition, a scale for weighing animals is desirable and strongly recommended. A restraint device or an area for restraint should be included in the design of every facility (Fouraker and Wagener, 1996).

Natural substrates should be spot-cleaned and raked daily, and hard-surfaced areas that are not exposed to the elements should be dry-cleaned or hosed daily (Fouraker and Wagener, 1996).

Where rhinos create an outdoor midden as wild rhinos do, this should be left in place. Care needs to be taken to ensure that this does not produce hygiene problems especially during wet periods.

Thick ropes coiled round tree trunks have provided adequate protection for large trees against horn damage with smaller trees needing protection from being pushed over.

Figure 2.8: Rope protecting large trees.

Figure 2.9: Large rocks protecting a small live tree.

2.2.4 Outdoor environment

The minimum outside air temperatures suitable for rhinos to be shut outside will vary considerably with weather conditions for example with wind chill, sunshine or shade etc. It is recommended that enclosures be designed such that animals may be kept outdoors as much as is possible. However, it is not recommended that animals are shut outside for long periods at temperatures < 10°C unless it is sunny and they have access to shelter. Considerably lower temperatures can be tolerated for short periods and many animals even enjoy playing in the snow! Generally it is not recommended to allow animals outside in temperatures less than -5°C even for a very short time.

2.2.5 Outdoor dimensions

It is difficult to recommend appropriate outdoor enclosure size as it will vary greatly depending on the topography, furnishings and configuration of the paddocks as well as the individual animals. A range of different paddock/outside space configurations is recommended to allow flexibility to cope with the variety of situations that will arise when managing a breeding group. For example a minimum area of 1000m^2 would be recommended for mixing animals, whereas all-weather outside yards of 500m^2 or more may be suitable for short term use by individual animals.

2.3 Feeding

An essential consideration in the welfare of zoo animals is to provide a good diet that meets the natural feeding ecology as close as possible. Nutrition takes a big role in longevity, disease prevention, growth and reproduction (EAZA, 2006).

2.3.1 Basic diet

As mentioned in paragraph *1.3 Physiology* the anatomy of the digestive system roughly resembles that of horses. That's why the nutritional requirements for horses and ponies are also used for the Black rhino. The minimum nutrient requirements are listed in the table below (Dierenfeld 1996).

Table 2.1: Nutrient concentrations in total diets for horses and ponies (Dierenfeld, 1996).

Nutrient	Growing	Mature/Maintenance	Pregnant/Lactating
Dig. Energy (Mcal/kg)	2.45 - 2.90	2.0	2.25 - 2.60
Crude protein (%)	12 - 15	8.0	10 – 13
Ca (%)	0.6	0.3	0.4
P (%)	0.3	0.2	0.3
Mg (%)	0.1	0.1	0.1
K (%)	0.3	0.3	0.4
Na (%)	0.1	0.1	0.1
S (%)	0.15	0.15	0.15
Fe (mg/kg)	50	50	50
Mn (mg/kg)	40	40	40
Cu (mg/kg)	10	10	10
Zn (mg/kg)	40	40	40
Se (mg/kg)	0.1	0.1	0.1
I (mg/kg)	0.1	0.1	0.1
Co (mg/kg)	0.1	0.1	0.1
Vitamin A (IU/kg)	2000	2000	3000
Vitamin D (IU/kg)	800	300	600
Vitamin E (IU/kg)	80	50	80

Hay: Rhinos are large herbivores that are adapted for gaining energy from the fermentation of fibrous plant material. Black rhinos can be fed only grass hay. If this is done, and the protein content of the grass hay is not being monitored by laboratory analyses, the addition of legume hay (also known as alfalfa hay) to the grass portion of the diet (20% of the grass hay offered) is recommended in order to ensure adequate protein levels. However, generally a mixture of 1:1 grass hay and legume hay is recommended for the Black rhino to mimic the nutrient composition of the natural diet. There is speculation that a high proportion of grass hay may lead to excessive tooth wear in Black rhinos (Taylor *et al*., submitted). There is no published evidence, but the exclusive use of lucerne hay for Black rhinos is discouraged. When freshly cut grass is available this could be fed as well but, when the grass is cut too short it can cause constipation of the hindgut (Clauss and Hatt, 2006). In studies of intake, digestion and passage in zoo herbivores, dry matter (DM) intakes of approximately 1% of body mass when Black rhinos were fed grass hays, and slightly higher levels (1.2 to 1.6% of body mass) when fed legume hay (Dierenfeld, 1996). According to Clauss and Hatt (2006) the maintenance requirements of hindgut fermenters should be 0.6MJ digestible energy per $kg^{0.75}$ metabolic body mass.

Browse: For browsing rhino species, the addition of fresh and / or frozen browse may be essential to dietary health. Browse may contribute required nutrients that have not yet been quantified and may also be of benefit to dilute a captive diet that is too digestible (Dierenfeld, 1996). Browse that could be fed to black rhinos are:

- Willow (*Salix spp*)
- Beech (*Fagus spp*)
- Hazel (*Corylus spp*)
- Ash (*Fraxinus*)
- Birch (*Betula spp*)
- Oak (*Quercus spp*)
- Hawthorn (*Crataegus spp*)
- Robinia (*Robinia spp*)
- Poplar (*Populus spp*)
- Apple (*Malus spp*)
- Cherry (*Prunus spp*)
- Prune (*Prunus spp*)
- Pear (*Pyrus spp*)
- Wild rose (*Rosa spp*)
- Blackberry (*Rubus spp*)

For feeding browse in the winter browse can be preserved by silaging (Clauss and Hatt, 2006). Browse should be fed 7 days per week. A browsing Black rhino can be found in Figure 3.

Figure 3: Black rhino browsing on fresh Hawthorn (*Crataegus* spp).

Concentrates: When feeding concentrates the pellets should be smaller than 1 cm in diameter for a proper intake of the pellets (Dierenfeld, 1996). The portion of pelleted compound feeds (or other forms of concentrates) in the diet should not exceed one-third of the overall calorific value. It should

be possible to deliver sufficient amounts of energy and protein while providing a substantially lower proportion of pelleted compound feeds or concentrates in the diet. Pelleted compound feed may be used to balance mineral, vitamin and in some cases protein requirements. Pelleted compound feeds should only be used to satisfy energy needs when adequate roughage is not available. A pelleted compound feed based on lucerne meal, with a high concentration of vitamins and minerals (except iron) is recommended so that only small amounts need to be fed. When pelleted compound feeds are used it is recommended that it has high-fibre content (crude fibre 20% and acid-detergent fibre (ADF) of 25% of DM) (Clauss and Hatt, 2006). The proportion of concentrates in the diet should be between 1 and 10%.

Supplements: A possible vitamin-E deficiency has been suggested but not confirmed in zoo rhinos; current recommendations based on natural browse composition suggest that diets should contain 150 to 200 IU vitamin E/kg dry matter (a value usually surpassed in pelleted compound feed). If grown in an area prone to soil selenium (Se) deficiency, forage should be tested routinely for determination of Se content to provide data needed for balancing rations (Dierenfeld, 1996).

Iron: Over supplementation of iron is of particular concern in Black rhinos because this can cause several uncommon diseases. The recommended amount of 50 mg iron/kg DM for horses will probably be exceeded by the hay mixes described, and also by most pelleted feeds used. The use of tannin might reduce the excessive iron absorption. But there is no quantitative proof regarding supplementation of tannin in captive rhinos. According to Clauss and Hatt (2006), who assessed the results of studies regarding the effect of tannin supplementation in other species, the iron absorption will probably reduce by increased dietary tannin content. Extra supplementation with iron is not recommended (Clauss and Hatt, 2006). One Black rhino collection uses supplementation of tannin.

Fatty acids: The supplementation of linolenic acid (n-3) could be necessary to balance the amount of linoleic acid (n-6) and linolenic acid (n-3). This could be done by feeding fresh forage like freshly cut grass and browse, by increasing the proportion of grass or lucerne hay in the overall diet, by using concentrates that are based on lucerne meal rather than grain or soy products or by including linseed or linseed oil in the concentrates (Clauss and Hatt, 2006).

Salt lick: Black rhinos have been found to have higher endogenous faecal sodium losses. To counter sodium deficiency salt licks (suitable for horses) should be available *ad libitum* (Clauss and Hatt, 2006).

2.3.2 Special dietary requirements

Calves: Rhino calves can be hand reared in captivity. Please refer to chapter *2.5.6 Hand rearing* for information about milk formulas, hand rearing and weaning.

2.3.3 Method of feeding

The concentrate portion of the ration should be given in at least two feedings daily for better utilisation. When practical, a small feeding of hay should be encouraged prior to each concentrate feeding (Dierenfeld, 1996). Feeding can either occur at a fixed time and fixed feeding place, or hidden around the enclosure.

Food should be offered on a concrete pad / livestock troughs / hay racks or simply a pile in the corner. Sand impaction has previously been documented in rhinos; therefore, feeding directly on the ground is not recommended. To reduce competition for food if animals are kept together, individual feeding stations or adequate space at communal feeders is recommended (Dierenfeld, 1996).

If Black rhinos have any problem with eating, animals can sometimes be encouraged to consume less palatable forages if hays are soaked in water or sprinkled with molasses.

Apple sauce had proved to be helpful in administering unpalatable medications and / or supplements (Dierenfeld, 1996).

2.3.4 Body condition scoring

To decide when to increase or decrease the amount of food, the body condition score should be used. Body condition scoring (BCS) involves the visual assessment of specific parts of the body for muscle and fat content, and can be a useful indicator of general health and condition of an individual. A standardised body condition scoring system has previously been developed for Black rhinoceros (Reuter and Adcock 1998), which assesses seven key areas of the body, ranging from BCS 1.0 (emaciated) to 5.0 (heavy). Ideally, a 0.5 point scale can be used to assess these areas, to give a representation of the relative condition of individuals. It should be noted that the Reuter and Adcock (1998) BCS was designed for wild living rhinos where they consider a score of 5 to be excellent. In zoos however we see overweight animals and therefore consider a BCS of 4 to be ideal.

Reuter and Adcock (1998) have made a list of criteria for each body condition score. These criteria can be found in Appendix 1 (Reuter and Adcock). Another way to assess the nutritional status of the rhinos is by regularly weighing the animals and recording this data (Clauss and Hatt, 2006). Weighing of the diet is recommended.

Body condition may be of relevance to reproductive performance, particularly in females, as recent research has indicated that non-breeding females tend to have higher BCS than breeding females (60% of non-proven females were scored 4.5 compared to only 6% of proven females) (Edwards et al. 2013).

It is recommended that a side view and rear view colour photograph (not taken in bright sunlight) are sent to Becca Biddle at Chester Zoo (b.biddle@chesterzoo.org) annually, to validate the body condition score.

A rhino with a good body condition score can be recognised by:

- The neck appears thick across the top, and is well muscled, with a smooth gradation between the neck and the shoulder blade.
- The shoulder (scapular region) is well covered, and slightly rounded but not bulging.
- The ribs (costal region) are covered with thick skin folds, especially just behind the shoulder and elbow region.
- The spine (vertebral region) appears rounded and the long back muscle and fat deposits fill the gap between the ribs and the spine.
- The bony points of the rump (gluteal region) are covered and the rump appears flat as opposed to rounded.
- The abdomen (abdominal region) appears filled and taught.
- The tail base should be rounded but not bulging.

In Figure 3.1 you can see the different body regions, and in Figure 3.2 the different body condition scores 1 – 4 are displayed (Reuter and Adcock, 1998).

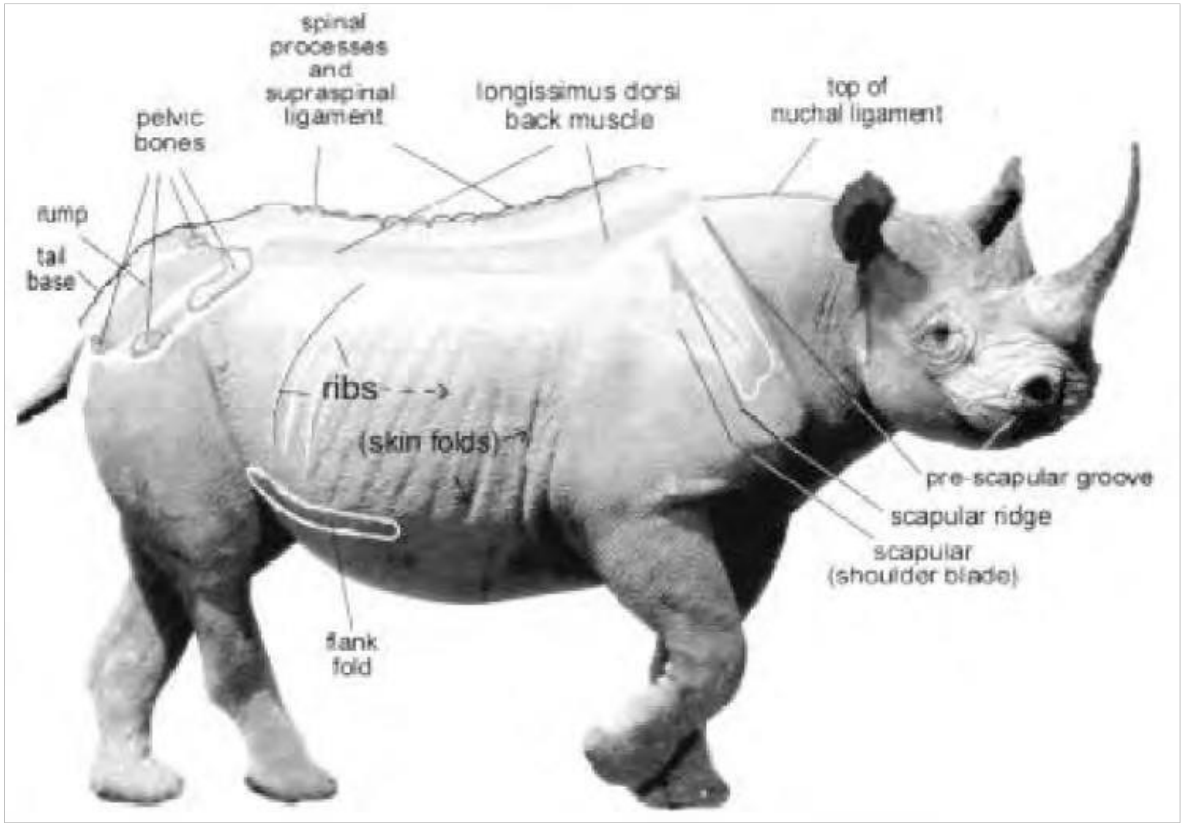


Figure 3.1: Black rhino body regions (Reuter and Adcock, 1998).

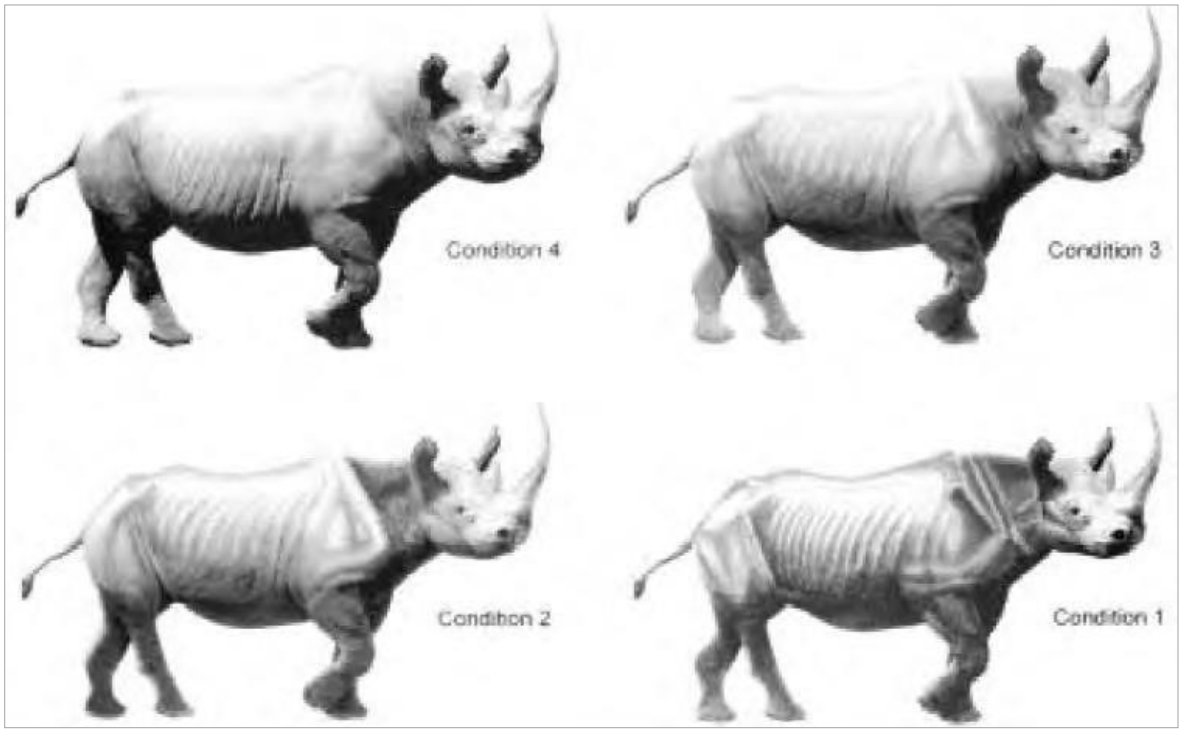


Figure 3.2: Black rhino body condition scores (Reuter and Adcock, 1998).

2.3.5 Water

Water should be freely available at all times (Dierenfeld, 1996; EAZA yearbook).

2.4 Social structure

The social organisation of the Black rhino in the wild is described in chapter *1.8.4 Social behaviour.*

The following chapter describes the social structure and introduction procedures in captivity.

2.4.1 Basic social structure

Black rhinos should be kept individually, in pairs, or in a trio of one male and two females. Keeping Black rhinos in larger temporary groups may possible particularly when young, however this varies between individuals and will depend on the size of the enclosure and the number of visual breaks. Two adult females together often works well but as all Black rhino groups they need careful observation.

The optimum group composition is 1.2. Since Black rhino males are territorial, each bull should have his own enclosure. Adult males in the same exhibit are not recommended and barriers must be

solidly built, with no visual contact. Adult females in the same exhibit may be possible. An adult male and an adult female in the same exhibit is often possible but may need separating at times. Allowing compatible individuals to spend as much time as possible with each other may prove beneficial. Almost all of the institutions holding Black rhinos house them in pair situations, although they have also been exhibited in trios (1.2). If this is being considered, animal managers should monitor the behaviour of the dominant female closely, as female suppression has been recorded in select cases (EAZA yearbook, 1995; Fouraker and Wagener, 1996; Rieches, 1999).

2.4.2 Changing group structure

When introducing rhinos it is important to provide auditory, olfactory and visual contact between the individuals. Tactile contact through bars should be provided. Introductions should be made after careful acclimatisation of the animals. Experienced staff members should always be present for introductions and a plan for separating them should be in place, should this be necessary e.g. high pressure water hose (EAZA yearbook, 1995). It may be necessary to keep the animals separately when they are inside at night.

Steps in the introduction process - *this applies to all introductions, the animals should be kept separated until step 4.*

1. Animals in the same indoor enclosure or multiple outdoor enclosures should have olfactory and auditory exposure to each other. If the animals are not housed near each other (i.e., enclosures on opposite sides of the zoo, etc.) they should be moved to the same exhibit area.
2. Animals should be given visual contact with each other in addition to the above sensory modalities. If at any point during this process the animals display symptoms associated with stress (e.g., pacing, diarrhoea, excessive vocalisation) for more than two to three hours, the introduction should return to the previous step.
3. If animals are not already positioned adjacent to each other, they should be moved closer together (e.g., to adjacent stalls or adjacent outdoor enclosures).
4. The actual introduction (full tactile exposure) should take place in the largest enclosure available. Preferably, the enclosure should be familiar to the least dominant animal and include ample “run-arounds”.
5. Within institutions in which rhinos can be left together 24 hours a day, they should be separated during the first several nights or until they show only minor aggression (Fouraker and Wagener, 1996).

Aggression in Black rhinos ranges from ritualised to true aggression. Face-to-face staring is often seen at the beginning stages of ritualised aggression and may be an opportunity for the participants to “size each other up”. Ritualised aggression may subsequently proceed to fencing or sparring and then charging with or without an open mouth threat. Aggression becomes more serious as oneanimal begins chasing the other, which also include or lead to horn strikes and gores. With this kindof aggression problems arise in small enclosures and in dry moat ditches. Another indicator for aggression is coming into oestrus. Excited animals run with their tail up and very often start to be aggressive (Fouraker and Wagener, 1996).

Tranquilisers are only recommended when aggressive animals are involved or / and when an animal is very nervous. Two experts reported experience with the use of tranquilisers during an introduction, there are different methods described in chapter *2.7.3 Catching / restraining.*

When introducing more animals together, at first start with the introduction of the females and with the male as the last animal to be introduced. The introduction of a male and a (post-partum) female is described in chapter *2.5.1 Mating*.

Introduction of a new female to an established male-female group: Female Black rhinos generally do not tend to form strong pair bonds. Therefore, a new female should be introduced to an established male-female group one individual at a time, but it is not necessary that she is introduced to all females before being introduced to the male (Fouraker and Wagener, 1996).

Introduction of a new male to an established female group: As previously discussed, female Black rhinos do not generally tend to form strong pair bonds. However, if a multiple-female group is established and managers perceive that the females have formed strong bonds, the new male should be introduced to the females as a group rather than to one female at a time. If the females are not compatible, but an introduction is necessary (EEP recommendation, breeding, etc.), the new male should be introduced to each female individually (Fouraker and Wagener, 1996).

Introducing a female to a female: Two females may be kept in the same enclosure depending on the characters of both animals. Initially, the females should be familiarised with the enclosure. The females should have contact through bars when they are indoors. When they no longer show aggression toward each other, the rhinos can be introduced. Close observation is necessary after the introduction (EAZA yearbook, 1995).

2.4.3 Sharing enclosure with other species

The critical factors for sharing the enclosure with other species are space and refuge availability, including visual barriers. In a non-breeding situation rhino species have been successfully mixed with birds and hoof stock. In all cases the dispositions of the individual animals, as well as adequate space and exhibit structure are important considerations prior to attempting a mixed species exhibit. Black rhinos have been reported to be problematic in mixed species exhibits. Both sexes have attacked and killed neonate and adult ungulates (EAZA yearbook, 1995; Fouraker and Wagener, 1996; Guldenschuh and von Houwald, 2002; Rieches, 1999).

Black rhinos can be mixed indoor with several small bird species. Another species mentioned is the ostrich however this mix has failed. Black rhinos can be mixed with (water) birds. According to the Rhino Keepers' Workshop 2001 Husbandry Survey one zoo, Disney Animal Kingdom, has a mixed enclosure involving Black rhino, Pink backed pelican (*Pelecanus rufescens*) and Yellow billed stork (*Mycteria ibis*) *(Mehrdadfar, 2002).*

Red-billed ox peckers (*Buphagus erythrorhynchus*) have been mixed with Black rhinos in an indoor exhibit. The rhinos and ox peckers were only mixed when the rhinos were indoors. During the day the rhinos were locked outside of the house. Other species present in the same exhibit were Double-toothed barbet (*Lybius bidentatus*), Violet turaco (*Musophaga violacea*), Cattle egret (*Bubulcus ibis*), African grey hornbill (*Tockus nasutus epirhinus*), and Dinemelli's weaver (*Dinemellia dinemelli*). The Black rhinos were highly intolerant to the ox peckers. The Ox peckers were feeding on the wounds of the Black rhino and they were making new wounds. Only half of the time the rhinos managed to chase the Ox peckers away. It is recommended observing both species when mixed (McElligott et al, 2004).

2.5 Breeding

Breeding success is related to the size of the zoo enclosures where courtship and mating occurs. This is important because of the quite aggressive pre-copulatory behaviour. The primarily requirement for successful breeding Black rhinos is pair compatibility. Research of Carlstead *et al*, suggests that "a compatible pair is one in which the female is relatively more aggressive and assertive, and the male more submissive and adaptable" (Carlstead et al, 1999).

2.5.1 Mating

Breeding success may be enhanced by separating males from females as little as possible. For general information about mixing Black rhinos please refer to chapter *2.4.2 Changing group structure.* Specific information about male and female introduction is described below. This followed by a description of behaviour for female and male in relation with mating.

Introducing a male to a female: It is often easier to introduce a male to a female when she is in oestrus. Some collections have reported that for some pairs it is better to introduce them before the female is in oestrous as the male may get too excited when introduced when the female is in oestrus.This is very dependent on the individuals involved. Some females do not express clear behavioural signs of oestrus, even if they are cycling regularly and so should in fact be receptive. This has been reported to be a particular problem in previously non-proven females (Edwards *et al*. 2013), where females may be cycling based on hormone data, but often do not express overt behavioural signs of oestrus. Hormone analysis can then prove useful by allowing prediction of the period of female receptivity, even if behavioural signs are relatively absent. When introducing a male to a female both animals should be familiarised with the enclosure. The introduction should occur in the largest paddock available, following the general introduction steps stated in chapter *2.4.2 Changing group structure*. If a single large paddock is not available, adjoining paddocks should be opened to form a large area for the introduction. If the latter strategy is used, care should be taken to modify any resulting dead ends in the exhibit where a rhino may become trapped during an aggressive interaction. This method is proven to be successful. If it is not the usual enclosure of the male, he should have been given time to mark the enclosure. This should not be cleaned out. Before the introduction the contact through bars (indoors) must be sufficiently long until no more aggression is

shown. Observation should continue when needed. In any case but especially when aggressive situations arise, preferred feed should widely be distributed throughout the enclosure (fresh greens, browse, carrots, etc) (EAZA yearbook, 1995; Fouraker and Wagener, 1996).

Introducing a postpartum female to a male: The reintroduction of a male to a female immediately postpartum is not recommended. If the calf is still born or does not survive it is recommended that after four months, reproductive hormone monitoring is resumed to determine the next oestrus. This combined with a judgement of the female's health dependant on the difficulty of the birth, can be used to decide when to reintroduce the male for mating. If the calf survives, it is recommended that reproductive hormone monitoring resumes by seven months to determine the next oestrus.

It has been reported in the wild female Black rhinos that cyclicity resumes as early as three months after parturition (Goddard, 1967). There has been one report from a captive female at Hannover zoo who was observed to be in oestrus 20 days following giving birth (Dittrich 1967), she was then reported as cycling regularly every 25 - 30 days (Goddard 1967) until conception approximately twelve months after giving birth. Hormone analysis on captive females by Brown *et al*. (2001) has confirmed that females generally resumed cyclicity within three to ten months post-partum.

If cycling occurs, and it is possible to separate the mother from the calf for a long enough period of time, the male can be reintroduced for mating when the calf is separated. The possibility of training the calf to be separated from the female after the age of seven months, to allow re-mating of the female, depends highly on the character and behaviour of both adult and calf, but can help to reduce the interbirthing period.

Studies have shown interbirthing periods in wild Black rhinos to be highly variable; from as short as 20 months, to 89 months, the mean interbirthing period of Black rhinos was 44 months in Hluhluwe National Park and 30 months at Umfolozi National Park (Bertschinger, 1994). In captivity the shortest interbirthing period reported is 16 months (Smith and Read 1992), indicating potential conception during first post-partum oestrus, however this is relatively rare.

The average interbirthing period in the current living EEP (Oct 2013) is 46 months (calculated from 40 separate cases where the female's previous calf survived more than four years). This is longer than seen in the wild. In almost half of these cases (48%) the interbirthing period was less than 40 months meaning the calf was separated at less than two years old to allow re-mating of the female. In 13%of these cases, the calf was less than 18 months when separated from the female to allow her to be re-mated. The shortest interbirthing period from these 40 cases was 26 months. The female in this case was 'Nane.' If Krefeld zoo, and if the gestation period was fully served, her calf must have been separated from her at ten months old to allow mating to occur. In Nane's case, short interbirthing periods are common; she has four surviving offspring born at intervals of 27, 26 and 36 months. This may be due to a combination of factors including her confident character, dominance over the male, and very peaceful mating (always the same male). Nane gives clear behavioural indicators that she has come back into oestrus between three and four months after parturition allowing the keepers to decide the best time to re-introduce the male.

Female behaviour: At the peak of oestrus the female shows the following behaviour: *positive male solicitation, presentation of hindquarters, aggression towards the male, running from the male, ignoring the male, copulation as well as refusing to copulate. Vulva changes include: squirting of white or cloudy urine, vulva swelling with occasional mucosal discharge prior to mating* (Fouraker and Wagener, 1996). At least two collections have succesfully used the hormone treatment *Regumate* to induce ovulation.

Male behaviour: Although, males in captivity may show interest in females outside of peak oestrus, greatest interest is seen during the peak of oestrus. At the peak of oestrus the male frequently exhibits the following behaviour: *erection, genital inspection and flehmen response, head resting, chasing, mounting, copulation, failing copulation attempts* (Fouraker and Wagener, 1996).

Ideally a male will show interest in a female one to three days prior to oestrus. The couple may need to be separated for the night. Copulation normally occurs the next day. Copulation will last 20 to 45 minutes with multiple ejaculations. Mating may occur for 24 hours (Fouraker and Wagener, 1996).

2.5.2 Reproductive endocrinology as a management tool

Using hormone analysis as an additional tool to manage introductions can be very useful, particularly in females where behavioural signs of oestrus are weak or unreliable. Non-invasive approaches are preferable as they minimise the disturbance to the animal and can allow long-term sampling as part of the keepers' daily routine. Faecal samples are often preferable to urine samples, as collection is often easier, and requires no additional training of the animal, instead fresh samples can be collectedwhen the animal is let outside first thing in the morning. Samples should be frozen immediately after collection, and stored frozen until shipping to a laboratory for analysis.

Samples collected at least every other day are necessary for characterising oestrous cycles in females, and can be used to determine a females' typical oestrous cycle length. This can then be used to predict when she will next be in oestrus, and therefore give keepers extra confidence when deciding when to introduce a pair (or trio) for breeding purposes. See Figure 3.3 for an example of results of hormone sampling to predict cycling. Samples collected on a weekly basis are sufficient to investigate differences in testosterone between males (see Appendix 2 for faecal sample collection protocol).

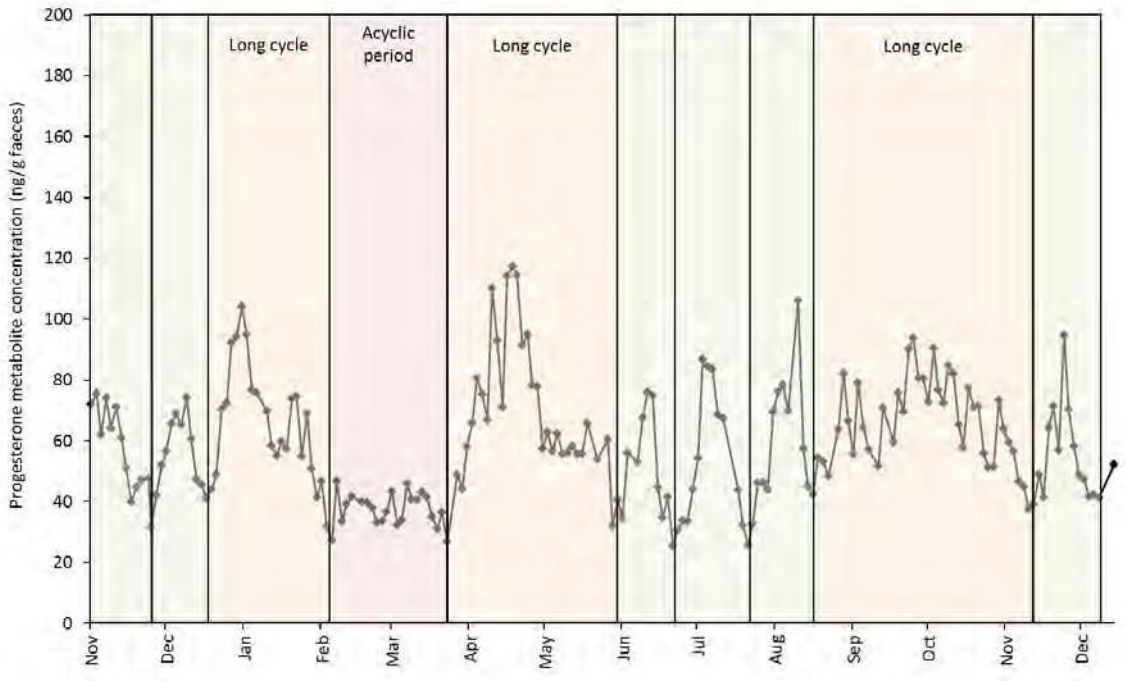


Figure 3.3: Progesterone metabolites (♦) measured in faeces collected from a female Black rhino can be used to characterise oestrous cycles. However, the length of oestrous cycles can be highly variable, with a normal cycle lasting between 20-40 days in length (green sections). However, shorter cycles less than 20 days (not shown) and longer cycles greater than 40 days in length (orange sections) are also relatively common, and periods of acyclicity (red section) are also observed.

2.5.3 Pregnancy

The gestation period is around fifteen to seventeen months, more information about the gestation period is found in chapter *1.7.5 Gestation period / birth rate.* It is recommended to test for pregnancy. Pregnancy testing can be done by faecal steroid sampling, urine steroid sampling, blood steroid sampling and by ultrasound. For blood sampling and using ultrasound the animal involved needs to be trained, while faecal and urine sampling involve less effort (EAZA yearbook, 1995).

Pregnancy diagnosis can also be performed using hormone analyses, samples collected every other day can be used to distinguish an increase in progesterone metabolite concentration, which occurs at around three months post-mating – patterns of progesterone secretion during this first three months are non-conclusive, and seem to show individual variation. Based on this progesterone metabolite increase, parturition can be approximated (see Figure 3.4 for an example of a female Black rhino hormone profile during pregnancy).

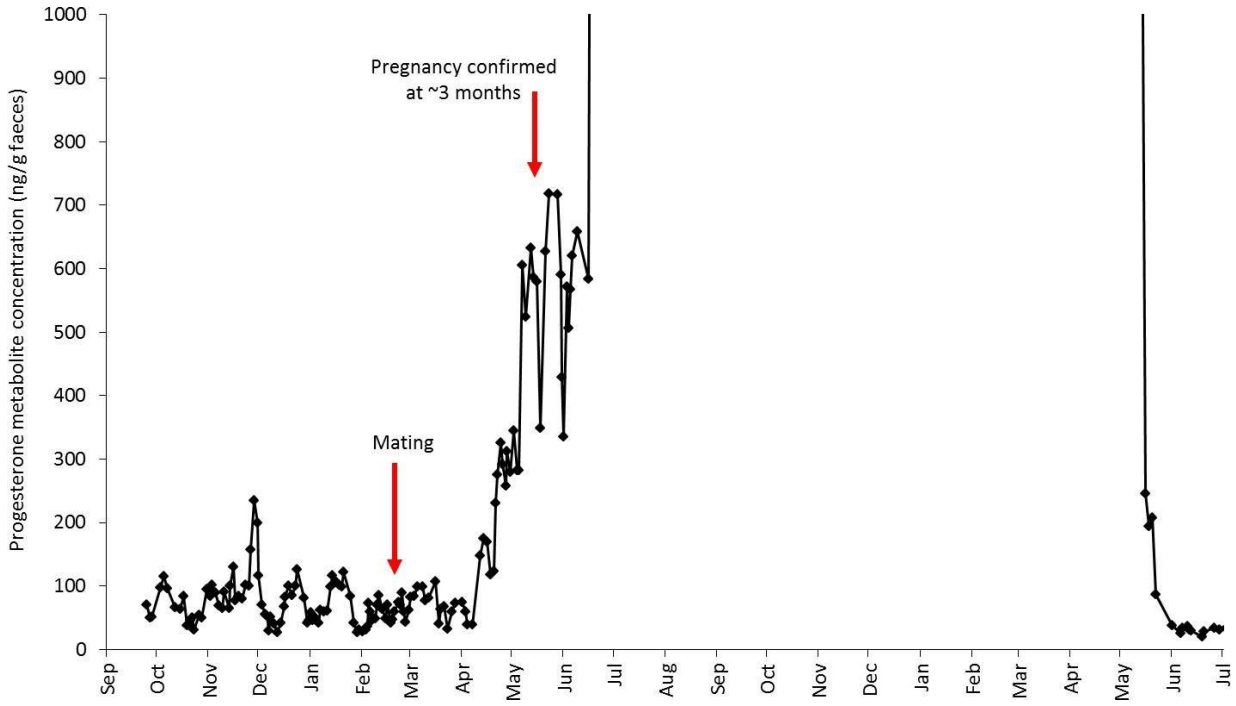


Figure 3.4: Example hormone profiles of a single Black rhinoceros pregnancy, in this case lasting 449 days. (a) Routine hormone monitoring was used to predict oestrus and time introduction to the male. After a successful mating it took approximately three months before pregnancy could reliably be confirmed, as individuals differ in their progesterone profile during this initial period.

2.5.4 Birth

The duration of delivery is short, usually lasting between one and three hours. Births often take place at night or in the early morning. It is not necessary to observe the birth, but if that is the case it is recommended to watch remotely on a TV monitor. Distocia is very rare. See Appendix 3 for an example birthing plan for female rhino *Ema Elsa* at Chester Zoo.

Indicators prior to birth:

- *Thirty days:* The female Black rhino teat size may increase and milk production begins. When pressure is put on the teats milk may be expended. It might be that the female prolapses vaginally when defecating (Fouraker and Wagener, 1996).

- *Two weeks:* The nipples of the female rhino may enlarge and the nipples develop wax plugs. The vulva will start swelling as well (Fouraker and Wagener, 1996).

- *Twenty-four to forty-eight hours*: The female Black rhino becomes irritable and aggressive to stimuli, including to the staff, also she may lose her appetite. The udder will increase dramatically in size; mucus plug forms and increased vulva dilation occurs. The female will lie

down more often and is restless. Other behaviour mentioned is frequent urination (Fouraker and Wagener, 1996).

Additionally, if daily hormone analysis is performed leading up to birth, a drop in progesterone metabolite concentration can precede parturition by 24 - 48 hours, allowing prediction of parturition in cases where this may be deemed necessary (Figure 3.5).

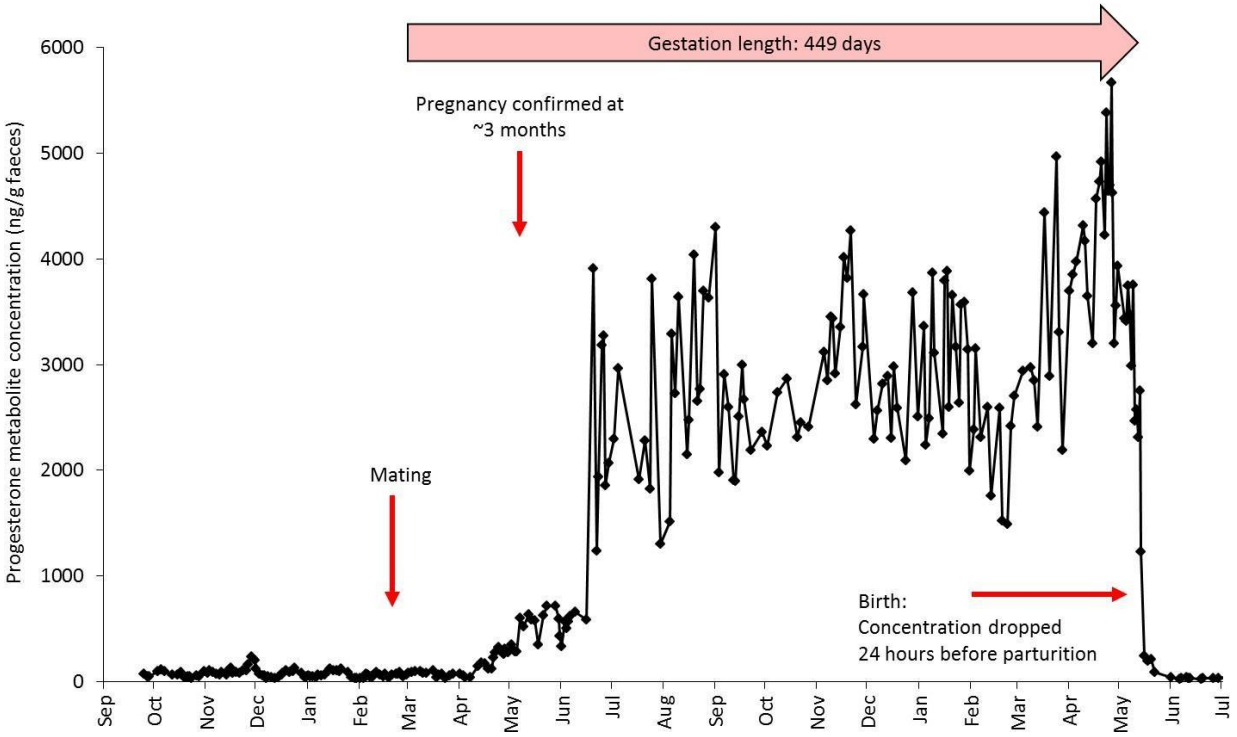


Figure 3.5: Example hormone profiles of a single Black rhinoceros pregnancy, in this case lasting 449 days. (b) At three months, progesterone concentration increases dramatically, and can be 10-20 fold higher than peak cycling concentrations. Parturition was predicted using hormone analyses as progesterone metabolite concentrations dropped 24 hours prior to parturition.

2.5.5 Development and care of the calf

Calving usually occurs during the night or the early hours of the morning. When the calf is born its weight is around 40 kg (Nowak, 1999). Immediately following birth, the new-born calf is usually cleaned by its mother. First standing up will be seen from 15 minutes to one or two hours after birth. A new-born calf may require traction material to help steady itself. Traction materials may include sand, gravel, straw, hay or rubber matting. These additional materials should be added well inadvance of calving to give the female time to get used to them. In all cases, both the dam and calf should be monitored closely but without disturbing them to allow them to bond. Monitoring using closed circuit television equipment may be preferable for a nervous or first time mother. A calf should begin suckling within one to two hours of standing. The mother will nurse the calf standing or lying on her side (Fouraker and Wagener, 1996).

A new-born rhino should be up and walking within approximately one hour after a normal birth. Following a dystocia, or breach birth, the dam may be too exhausted to clean and care for the calf

immediately. Similarly, calves weak from a dystocia may also take longer to stand. This, however, does not mean that intervention is necessary. It is advised to keep close monitoring. Suckling should be seen within the first five hours and should be frequent. While sleeping the calf should be with the dam constantly and touching her. A problem is indicated if the calf is seen alone for extended periods, appears weak, or is having trouble keeping up with the dam. If a problem is suspected and the dam and calf can be safely separated, daily weights of the calf should be obtained. Normal daily weight gains are representative of nursing success. When calves are under optimal weight, they should get supplemental bottle feedings. For bottle feedings check chapter *2.5.6 Hand rearing*. The faeces should be grey or yellowish grey in colour and the consistency of stiff putty. A healthy calf maynot have a stool for the first 24 to 48 hours. For all calves with diarrhoea, a faecal sample should be submitted for culture of enteric pathogens and internal parasite screen. Daily observations of the calf's stamina are important, as its condition may decline rapidly. Any deviation from the normal body temperature of 36.9 -37.8^{o}C is likely an indication of poor health (Gage, 2002).

A Black rhino calf should be separated from the mother between the ages of two and four years. It should not be sooner than 18 months.

2.5.6 Hand rearing

Hand rearing should only occur if absolutely necessary.

There are successful experiences with the hand rearing of Black rhinos. Hand rearing is only recommended when there is no other possibility. The infant should always remain with the mother and (when needed) be additionally bottle fed. Hand rearing could become necessary when the young is rejected by the mother or medical problems with the mother and / or infant exist, or when the infant fails to nurse. The hand rearing of an infant has to be considered very carefully and intensive care of one or two keepers will be needed (EAZA yearbook, 1995).

Record keeping: Accurate record keeping is extremely important. Maintain a daily log of formula intake, body weight, body temperatures, and faecal output and consistency. It is helpful to have this information in a format such that one month's statistics may be viewed on one page. Note exercise times and behavioural changes each day. Rhino calves should urinate large amounts daily without the assistance of physical stimulation. Weigh the calf at the same time each day for two to four weeks, every other day until two months of age, twice a week until three months of age, and once a week until five to six months of age. Weights may be obtained by leading the calf onto a platform scale while it is nursing on a bottle (Gage, 2002). All information should be sent to the EEP coordinator.

Equipment: The following items should be on hand:

- Pliable, one litre polyethylene laboratory bottle with a narrow mouth
- Artificial lamb's nipple with a crosscut opening
- Calf bottles and calf nipples
- Large containers with screw tops for storing formula
- Large cooking pot, hot plate, large refrigerator, sink, disinfectant and bottle brush

- Measuring cups, gram scale, walk-on platform scale
- Large stuffed animal to serve as a companion
- A radiant room heater, or other safe heating system, and an electric blanket may be needed (Gage, 2002)

Housing: For a healthy calf the air temperature should be between 15-30^{o}C. If the air temperature is expected to drop below 15^{o}C, for example during the night, a heater will be needed. When the calf is hypothermic or debilitated a constant temperature of 26-30^{o}C is recommended. For bedding substrates like soft hay should be used. Wood shavings should be avoided due to possible intake, when the calf lies with its lips on the ground (Gage, 2002).

For mental and physical development a large exercise yard is important. A healthy new-born should be walked for half an hour, twice a day. The first few days the calf is allowed to explore the yard onits own with a keeper nearby for emotional security. A rhino calf loves to run and will do so after three-four days of age. This daily exercise encourages normal defecation (Gage, 2002; Wagner and Edwards, 2002).

For comfort and companionship a large stuffed animal may be placed with the calf. If another large ungulate neonate is available, it may be placed with the rhino calf after it has reached one week of age, replacing the stuffed animal. When there is no neonate ungulate available a young or adult sheep or goat can be used. Developing the bond between the two animals might take a week. First when one of them is nervous, it may be necessary to separate them during the night, putting the stuffed animal back in with the rhino calf. A companion animal also discourages the rhino from becoming dependent on keepers for security and companionship (Gage, 2002; Wagner and Edwards, 2002). Before introducing any companion animals it is essential that you know the full health history of the animal and the group it came from. Also, the animal must be healthy and parasite free. It is recommended that you check with your own veterinary advisor before any introduction to ensure that disease is not transmitted from the companion animal to the young rhino.

Toys should be provided at an age of one or two weeks. The toys should provide the calf to exercise its natural behaviour of head butting. It is important that the animal does not practise this behaviour on the keepers, due to potential danger, this behaviour should be discouraged. Suitable toys are: two electric cart tires bolted together in order to keep them upright and rolling, large plastic trash cans, boomer balls, or any other object which can be pushed around without the risk of the young rhino wedging its head inside the object (Gage, 2002).

Milk composition and formula selection: Based on available data, rhinoceros milk is more dilute than milks of other ungulate species. It is low in solids, low in protein, very low in fat, and high in sugar compared with milk of equids, bovids and cervids (Dierenfeld, 1996).

Though rhinoceros' milk is different from cow's milk, the latter may still be appropriate for hand rearing rhinos if used in combination with other ingredients, like extra iron, vitamins and lactaid. There is also special artificial milk available, and horse milk can be used as well. Cow's milk is low in iron; consequently, an iron source should be added to the formula at two drops per 100 g of formula.

In addition, infant vitamins should also be added to the formula at two drops per 100 g of formula. Some infant vitamins may contains added iron.

The animal may also benefit from the addition of lactaid at one drop per 100 g of formula. Lactaid aids in carbohydrate digestion and helps prevent possible gastrointestinal distress *(Dierenfeld, 1996).* If the neonate is less than 24 hours old, colostrums diluted 50% with water or an electrolyte solution for ungulates, such as Replenish, should be administered for the first 24 hours. Though species- specific is preferred, cow colostrums may be used. To avoid gastrointestinal distress, a diluted formula may be offered beginning on day two. The formula can be gradually increased to fullconcentration depending on the animal's health, including weight gain and stool condition. Table 2.2 shows an example for formula and feeding regime used in San Diego Wild Animal Park. This method was successful for hand rearing Black rhino calves (Dierenfeld, 1996; Gage, 2002).

Table 2.2: Rhino formula and guidelines used at the San Diego Wild Animal Park (Gage, 2002; Wagner and Edwards, 2002).

Age	Formula	Ratios	Feedings per day[1]
1 day old	100% Cow's colostrum		7 times, every 2 hrs
2 days old	NFC:LFC:Lactose:H_2O w/ 50% colostrum	27:9:1:1[2]	7 times, every 2 hrs
3 days to 1 month Early lactation form.	NFC:LFC:Lactose:H_2O w/ 10% colostrum	27:9:1:1	7 times, every 2 hrs
1 to 3,5 months Early lactation form.	NFC:LFC:Lactose:H_2O	27:9:1:1	5 times, every 3 hrs[3]
3,5 to 6 months Mid-lactation form.	NFC:LFC:Lactose:H_2O	27:9:1:2	4 times
6 to 9 months Mid-lactation form.	NFC:LFC:Lactose:H_2O	27:9:1:3	3 times
9 to 12 months Mid-lactation form	NFC:LFC:Lactose:H_2O	27:9:1:4	3 times
12 to 15 months Late lactation form.	NFC:LFC:Lactose:H_2O	27:9:1:6	2 times
15 to 16 months Late lactation form.	NFC:LFC:Lactose:H_2O	27:9:1:8	2 times

Note: NFC = liquid non-fat cow's milk (skim milk); LFC= Liquid low-fat cow's milk (1% fat); lactose powdered, edible grade dextrose (reagent grade) may be substituted for the lactose.

[1]Day consists of a twelve-hour period from 6 a.m. to 6 p.m.
[2]27 parts NFC to 9 parts LFC to 1 part Lactose to 1 part water
[3]At roughly two months of age the calf can go to 4 times per day.

Feeding regime: Hygiene is very important in order to avoid contamination of the milk. The calf should be preferably bottle fed instead of feeding with a bucket to avoid hasty drinking. Fresh water should be available at all times.

Quantity fed should range from 10% to 13% of body weight. Rhinos do not need to be fed around the clock. Animals should be fed every two hours. Because infants suckle during daylight hours, feeding should be equally spaced in a twelve hour period not to exceed 3% of body weight at any onefeeding. It is recommended that feeding begin with 10% of body weight split equally into seven feedstwo hours apart during daylight hours.

The quantity of formula fed should be adjusted daily based on the animal's weight. Animals should be weighed at the same time each day. During the first weeks feeding should also be during night, with an interval of two to three hours. If diarrhoea occurs, the quantity of formula fed should be decreased or the formula diluted until stool condition returns to normal. If diarrhoea is persistent, an electrolyte solution can be used to dilute the formula, replacing some or all of the water. In addition, the number of feedings can be increased to lessen the quantity fed at any one time.

Formula can be prepared ahead of time and warmed as needed. Water should be boiled to decrease possible contamination due to pathogens, and then refrigerated before being added to the formula. The formula should be refrigerated and used within 72 hours.

Prior to feeding, the formula should be warmed to the animal's body temperature. Calf nipples work well. Bottles should be boiled before use. Diluted bleach may be used as a disinfectant. Formula left over from each feed should be discarded (Dierenfeld, 1996; EAZA yearbook, 1995; Gage, 2002).

Weaning: Weaning may begin as early as six months and should be completed in one year. Weaning is a slow process involving carefully monitoring body weight and solid food consumption. Animals should have access to solid food at all times.

A nutritionally complete pelleted diet such as Calf Manna, horse feeds or high fibre ungulate pellets, in addition to alfalfa hay, is appropriate. Formula may be decreased by gradually elimination the number of feeds or decreasing the amount offered per feed and gradually decreasing the number of feeds (Dierenfeld, 1996; Gage, 2002).

2.5.7 Population management

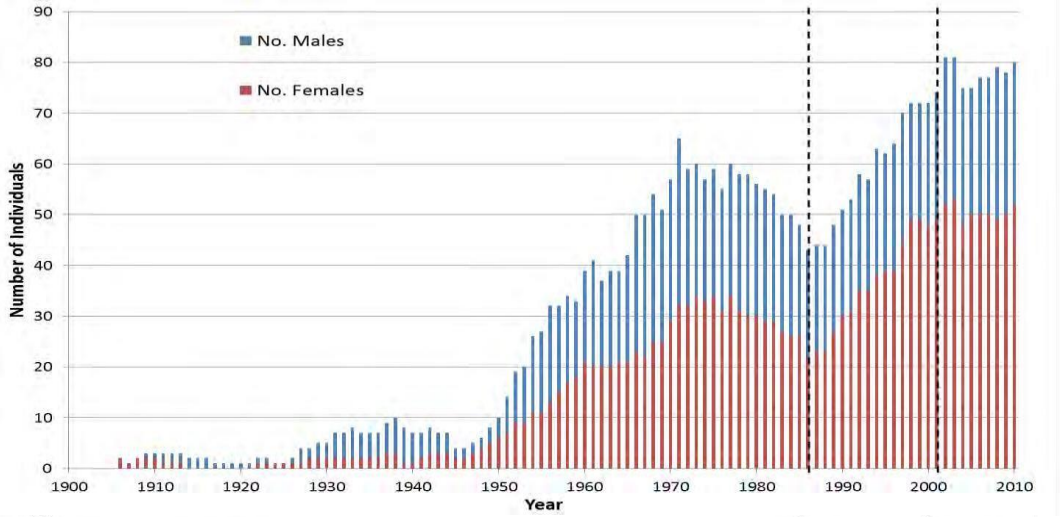
There is an international studbook for the Black rhino. In Europe there are only two subspecies of the Black rhino in captivity, the eastern Black rhino (*Diceros bicornis micheali*) and the south-central Black rhino (*D. b. minor*). The Regional collection plan of the EAZA rhino TAG from 2002 recommends the eastern Black rhino for EEP management. The south-central ssp. is not recommended to keep. The target population for the eastern Black rhino is 65/100/100 over 10, 50 and 100 years period. The EEP coordinator will determine the need for new specimens for the region. Other breeding programmes for the Black rhino are managed by AZA (SSP) and JAZA (SSCJ) (Lindsay, 2002; Foose and Wiese, 2006).

2.5.8 Sustainability of the EEP population

A recent EEP supported research project established to investigate population performance in Europe has used studbook data to predict the future growth of the population (Figure 3.6). These data suggest that unless reproductive output is increased, growth of the population is projected to be between one to two percent per year. In comparison, average growth rates of free living Black rhinoceros populations are around five percent per year, a target that could be achieved in the zoo population if reproductive output could be increased.

In the zoo population, average age at first reproduction is slightly later, and average inter-birth intervals (IBI) and are slightly longer than in the wild, but due to management constraints in captivity these may be difficult to reduce dramatically. However, the two main factors limiting population growth in Europe are a low proportion of females breeding per year (11.3% 2001-2010 and 15.7% 1986-2010 compared to 23.7% in the wild), and an unequal reproductive skew, with 42.1% males age 7-32 and 48.6% females age 5-32 yet to reproduce as of 31st December 2010. This latter factor could also have an impact on the genetic diversity of the population in the long-term.

a)



b)

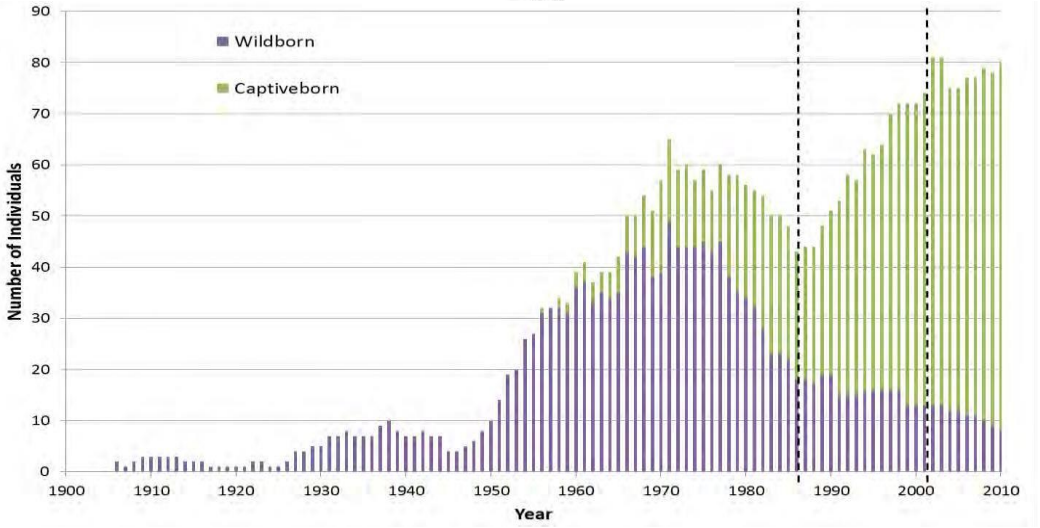


Figure 3.6: Census of the EEP Black rhinoceros population from studbook records indicating a) the number of females (red) and males (blue), or b) the number of wild caught (purple) and captive born (green) which make up the total population size each year. Dotted lines indicate the last 25-year and last 10-year time periods, where growth appears to have slowed.

2.6 Behavioural enrichment

Providing captive animals with opportunities to display a range of species appropriate behaviours and to make behavioural choices that give them some control over their lives is among the goals of an animal husbandry approach known variously as behavioural enrichment. Enrichment research in both laboratories and zoos has shown the importance of providing animals with an ever changing or rotating array of stimuli and behavioural opportunities (Ben-Ari, 2001). It is important that environmental enrichment encourages natural and not unnatural behaviours.

Enrichment may serve various functions like (1) improving well-being by reducing the levels of abnormal and injurious behaviour, increasing exercise, satisfying behavioural needs and optimizing the level of stimulation that animals receive, (2) educating zoo visitors by increasing the levels of natural and interesting behaviours, visibility and activity levels and (3) conserving endangered species by improving the success of captive breeding and reintroduction programmes. A simple way of behavioural enrichment is variability in enclosure topography and vegetation. Because rhinos can be aggressive towards each other, planting (protected from rhinos), rock piles, dirt mounds and other forms of visual barriers (mentioned in chapter *2.2.1 Boundary*) may help ease social tension by partially blocking rhino sightlines. Mud wallows and rubbing posts are other simple enrichment items and are particularly important for skin health (Fouraker and Wagener, 1996).

Designing indoor holding so that each rhino must pass through a common area prior to its individual stall allows rhinos to consistently sniff and mark another's dung-piles. Within reason, it is recommended that dung piles not be totally removed during cleaning. This again allows rhinos to obtain information about each other using their well-developed olfactory ability. With vet and management approval, dung may be exchanged with other zoos and placed in the rhino enclosure. The novel dung may stimulate sexual activity or increase territory marking behaviour. If the institution houses more than one rhino species or subgroups the same effect may be obtained by exchanging dung in-house (Fouraker and Wagener, 1996).

Rhinos may also benefit from addition of various objects. Enrichment items must be designed with the following criteria in mind:

- Can not be swallowed
- Can not be torn or ripped
- Can not be crushed or broken
- Can not trap or entangle the animal
- Can not cut, poke or scrape the animal

Objects that can be used as enrichment items are listed in table 2.3.

Table 2.3: Enrichment items (Felts, 2007; Fouraker and Wagener, 1996).

PVC Pipe	Spool with treats	Barrel
Waterbath (pool)	Moose stool	Bamboo contraption
Boomerball	Bison stool	Logs
Hanging boomerball	Pronghorn stool	Hanging logs
Street brush	Spinna	Sprinkler
Keg	Top soil	Stumps
Spool	Smashed barrel	Browse
Complex enclosure	Rubbing posts	

Another form of enrichment is having miscellaneous food items hidden in the substrate. By varying the time and location of food this will help to keep the animals occupied. Be sure to hide food on hard surfaces that are covered with mulched branches to avoid ingestion of sand or pebbles while the animals are digging for the hidden items (Gulenschuh and von Houwald, 2002).

An old tyre could be used as an enrichment item as well, it should be cut open so that the tire cannot get stuck around the nose, horn or neck of the rhino.

2.6.1 Training

There are several learning principles, namely positive reinforcement, negative reinforcement, "positive" punishment and negative punishment. We refer to Gatz (1998) for more information about these principles.

Training or operant conditioning programmes may also serve as a form of enrichment. Numerous examinations, as well as numerous nutritional, reproductive and veterinary research projects, often require hands-on contact with rhinos. An alternative to manual or chemical restraint of an individual is an operant conditioning programme that utilises positive reinforcement. Such a programme has many benefits, including reduced stress to the animal, more reliable sample collection, reduction of any effects of stress on the samples and less need for structural modifications to restrain animals. There are several institutions that have successfully trained rhinos to do procedures like blood collection, ultrasound and skin and food care (Fouraker and Wagener, 1996).

Before beginning any training the first step is to establish training programme goals and requirements. Training rhinos will require much coordination among staff members including keepers, curators, veterinarians and zoo management. Exhibit schedules may be modified during training. All parties must understand that consistency in routine is inevitable for training. Modifications will undoubtedly be made to the pre-training routine to accomplish the training programme goals (Fouraker and Wagener, 1996).

When training programme goals and requirements have been established the training of the animals can begin. The training process will generally include three basic steps:

- habituating of the animal to the trainer
- constructing and introducing targets, or visual areas of ideal placement for the rhino
- establishing the commands necessary to steer the animal to these target areas

It is recommended that training starts with one individual as the primary trainer. Additional personnel may be included once the rhino reliably executes the desired behaviours of the primary trainer. The goal is that ultimately, given the appropriate stimuli, the rhino will execute the desired behaviours for a number of different personnel. It is recommended that the training initially be performed in a specific area of the enclosure, but later on, flexibility is important so that the rhinowill perform the desired behaviours in more than one area if necessary. Training commands, targets and rewards should only be used during training sessions. Training commands and targets should be carefully evaluated prior to beginning the training programme. Most used commands in training sessions are: (Fouraker and Wagener, 1996)

- Move up; when a rhino needs to move forward
- Back; when a rhino needs to move back
- Over; when a rhino needs to do a side step
- Steady; when a rhino needs to hold its position
- Foot; when a rhino needs to present its foot
- Come; when a rhino needs to approach the trainer
- Target; when a rhino needs to place is head or body part at a specific area (e.g. on the target)
- All right; when the training is over and the rhino can do what it wants

Specific training areas and objectives will vary across institutions. Closed stall, free-stall and chutes work well for medical procedures, provided there is ample access to the animal and the safety for personnel (Fouraker and Wagener, 1996).

To habituate the rhino to the presence of the trainer, regular ten minutes training sessions may be effective. It should be emphasised that the amount of time required will depend on the tractability of the individual. The primary objective of these sessions is to establish trust. By noting generalised behaviours and body positions of the animal, the trainer should be able to notice when the animal is relaxed in the trainer's presence. At this point the trainer can begin shaping the desired behaviour. Each successive approximation of the desired behaviour should be rewarded with a command like "good", which serves as a bridge to link the behaviour to the reinforcement, which is given concurrently. A positive reinforcer should increase the frequency of the desired behaviour. Successful reinforcers are food (e.g. apple, bananas or bread) and, to a lesser extent, tactile stimulation (e.g. belly scratching). The bridge and reinforcer should only be given for the approximation of the desired behaviour. Otherwise, additional behaviours performed in conjunction with desired behaviour will also be reinforced (Fouraker and Wagener, 1996).

After successful completion of the approach behaviour the trainer can introduce a target, or object easily visible to the rhino. At this point the trainer should encourage the rhino to the target on command using the same basic procedure of reinforcing approximations of the desired behaviour. At this point training sessions should last about 10 to 30 minutes. Alignment with both head and body targets places the rhino in position for all kind of procedures like blood collection or rectal temperature readings. When the rhino successfully targets the target the next step is to encourage the rhino to remain stationary for a given period of time (using the command steady). Once these behaviours have been established the final step is to desensitise the rhino to medical equipment. Additional personnel who will be performing the procedure can be introduced to the training. Initially the collection area should be manipulated (e.g. touching and pinching or cleaning the colon of faeces). Any medical materials that will be used should be slowly introduced. These introductions should continue until the rhino shows no reaction to the equipment. The final stage prior to the actual procedure may include pressure from a blunt needle or insertion of a reproductive probe until the rhino shows no reaction (Fouraker and Wagener, 1996).

If at any point during the training there is regression, the trainer should revert to a point in the training where the rhino is comfortable and then slowly proceed again. This may add time to thetotal time needed for conditioning but the probability of the overall success is increased. Once the procedure is routine for the rhino the trainer should periodically lead the rhino in performing the desired behaviours if they are not otherwise performed regularly. In the absence of regular performance, this variable reinforcement will help prevent the behaviours from extinguishing (Fouraker and Wagener, 1996).

2.6.2 Crate training

Crate acclimation can require two to six weeks, however some institutions have reported that they have successfully crate-trained their rhinos in seven days or less. Training should be completed by a method of approximation.

The first step is to introduce the crate into an interactive part of the animal's environment, for example the door way, allowing the animals to go in and out and get used to it. Place the rhino'sfood in the crate to encourage this behaviour. Gradually introduce the front metal bars of the crate, and move the rhino's food gradually closer and closer to these, getting the animal used to them.

If the animal acclimates to the point of completely entering the crate and will allow the door to be shut, the door should be left closed for short acclimation periods under close observation, however this can take a long period of time (longer than six weeks) so it is recommended just to close the door shut on the actual day of transport. If the rhino does not completely acclimate to entering the crate, partial immobilisation (standing restraint) may need to be utilised for shipping. In situations in which crate training is not possible, immobilisation should be incorporated. Forced crating without training or immobilisation is strongly discouraged (Fouraker and Wagener, 1996).

2.7 Handling

2.7.1 Individual identification and sexing

Recommended methods for identification are documentation of characteristic marks like wounds and scars, and the use of microchips. Individual traits can be documented through photographs. If an animal is transferred, these records, or copies of them, should go with the animal to the new facility and the EEP coordinator. Microchips are also recommended as a primary identification method. They should be placed behind the left ear. Transponder identification numbers need to be reported to the studbook keeper (EAZA yearbook, 1995; Gulenschuh and von Houwald, 2002). Recommended method of sexing is by visual means.

2.7.2 General handling

Black rhinos can be kept hands-off or hands-on depending on the facility's policy and personality of the rhino. Preferably Black rhinos are maintained to allow a day-to-day, set-routine interaction whichwill facilitate medical and foot care, introductions, births and separations. It is not recommended that keepers enter the same enclosure space as Black rhino and that handling occurs through a barrier the gives the handler protection (Figure 3.7). For information about safety precautions we refer to paragraph *2.7.5 Safety*.

Figure 3.7: Handling should occur through a barrier ensure keeper protection at all times.

2.7.3 Catching / restraining

For physical exams as well as nutritional, reproductive or veterinary research projects physical restraint devices can be very valuable. Numerous institutions have constructed permanent physical devices to restrain their rhinos when necessary. These physical restraint devices are also called

chutes. In general, it is highly recommended that institutions modifying rhino exhibits or constructing new ones incorporate a physical restraint area or device into their design considerations. Several physical restraint designs are effective for rhinos. In general, major restraint chute design considerations include strength, durability, type and function. It should be noted, that available space and animal's size and disposition vary across institutions and should be individually addressed. Both captive managers and researchers emphasise that the general restraint area should be an active component of daily rhino management. Methods to accomplish this vary. A restraint chute or restraint area can be designed so that the rhinos must pass through it to exit the barn into the yard.If rhinos are fed indoors, part of the feed can be offered in the chute area. Rhino chutes should be manufactured out of steel or a combination of steel and steel-reinforced wood. Steel-strength aluminium has also been used. Aluminium is lighter and more manoeuvrable than steel, as well as potentially less stressful to rhinos because of lower sound properties than steel (Fouraker and Wagener, 1996). It is important to give the rhino time to get used to the chute so that it is calm and relaxed while being restrained. Depending on the temperament of the individual this may take many months to accomplish.

Restraint chutes: Permanent pass-through indoor restraint chutes are especially effective for rhinos. The chute should allow restraint of the animals when it is passing through in either direction so that shifting routine of the animal is not interrupted. The width of the chute should limit side-to-side movement while still allowing the animal to comfortably lie down. However, animals can become wedged in tight-fitting chutes if the side cannot be released. To alleviate excessive forward movement of the animal when it lowers its head, two vertical bars that push in from sides of the chute to the shoulder of the rhino may be utilised. Quick release of these shoulder bars often relieves agitated animals without having to release them completely (Fouraker and Wagener, 1996).

High-walled chutes: High-walled chutes or bars over the top keep the animals from climbing or rearing up. Horizontal bars in the chute's entry gates and sides are hazardous for examiners when the animal lies down. Vertical bars on the sides can trap researchers' arms if the animal can move forward. If the animal's movement forward and side-to-side mobility can be limited, vertical bars or walls on all sides are recommended. The distance between these bars along the sides of the chute should be great enough to prevent the animal's foot from becoming wedged if the animal rolls on its side in the chute. For researcher safety, this distance can be divided with removable vertical bars (Fouraker and Wagener, 1996).

Closed chute: A closed chute is another option that has been used successfully. A typical closed chute has both front and back gates. The back gate restricts the rhino's movement by sliding forward. The hind end of the rhino is supported by a v-design that prevents it from lying down. This design also allows additional safety for the staff while working with the animal. In many respects, a closed chute does not depend as strongly on conditioning of the rhinos as does a squeeze chute, though acclimation is recommended prior to attempting any treatments within the chute. The designof a closed chute might necessitate an outdoor location in most cases, thus the use of this type of chute may be limited by weather (Fouraker and Wagener, 1996).

Free-stall chute: A free-stall chute can be used for animals more sensitive to a confined enclosure. The design of this type of chute allows the rhino to enter or exit at its will and thus may help to keep

rhinos calmer during procedures. Because there is free access rhinos must be conditioned to target or stand still. A free-stall design can easily be incorporated into an existing pen or stall, indoor or outdoor. As stated, the open back of this type of chute allows the animal to enter and leave the structure at will. Protection of staff when working with the rhino is important, and a partial back wall constructed of vertical pipes allows staff to step out of the way (Fouraker and Wagener, 1996).

Sliding gates: Sliding gates are safer than swinging doors because rhinos may slam swinging doors. A rectangular opening in these gates for performing palpation should not pin the arm of an examiner when the animal is shifting. The distance between the vertical sides of this rectangular opening must be wide enough for researcher safety while still limiting the space through which a rhino could squeeze. Also the horizontal bottom bar of this rectangle should be only a few inches from the ground, as animals frequently lie down. Solid doors on the outside of these gates can be used to stop rhinos, as they may attempt to charge even small openings. Additionally, good lightning and accessible electrical sources are useful (Fouraker and Wagener, 1996). Guillotine gates are not recommended.

Immobilisation: Besides early crate training prior to transportation, immobilisation offers a fairly simple way of crating a rhino. The usual pre-immobilisation should be observed for any procedure requiring the use of chemical immobilisation/tranquilisation agents. For rhinos, etorphine (M-99, Large Animal Immobilon) remains the drug of choice although several alternatives are available. The duration of immobilisation without administration of an antagonist may range from 30 minutes to two hours (Fouraker and Wagener, 1996). For more information about specific drugs and immobilisation we refer to section *2.8 Veterinary* (Fouraker and Wagener, 1996). Other drugs used for immobilisation in combination are butorphanol, detomidine, xylazine and ketamine.

Following crating, all rhinos should be held for 24 hours at the loading location for observation, or accompanied by a veterinarian during transport. This is necessary because renarcotisation is common in hoofed animals, especially rhinos, given opioids. Trained personnel should be present to administer the correct reversal agent(s) in the likely event of renarcotisation. Any other complications of crating can be managed more easily and effectively in-house rather than en route (Fouraker and Wagener, 1996).

Several principles should be followed to increase the safety of chemical restraint procedures for both the animals and personnel:

- When applicable, antagonists to the restraint drugs should be prepared prior to the initiation of the procedures and should be available for rapid administration.
- Careful monitoring of the patient (auscultation, ECG, pulse-oximetry, etc) will help to rapidly identify problems should they develop and allow early intervention.
- The large size of an adult rhinoceros may result in further complications during anesthetic procedures. Efforts should be made to maintain the animal in sternal recumbancy when possible to minimize respiratory complications, and if the procedures is to last more than 30 min. efforts should be made to “pad” the area under the animal (with mattresses, inflated inner tubes, straw/hay bedding, etc.) to minimise the effects of pressure on the limbs (Fouraker and Wagener, 1996).

The kind of catching/restraining that is preferred is physical restrain or chemical restrain. When a rhino is being caught/restraint risk of injuring the horn needs to be taken into account.

2.7.4 Transportation

It is recommended to transport Black rhinos by truck or airplane in a crate. Each method has its advantages and each should be carefully considered and evaluated concerning the distance to be travelled, the personnel needed and the temperatures to which the animals will be subjected (Fouraker and Wagener, 1996).

Typical problems that can occur in shipping include the following:

- Animals destroying and or climbing out of the crate top
- Animals becoming inverted in the crate and unable to right themselves
- Animals destroying end panels or doors, resulting in eye, horn or facial injuries
- Prolonged excessive exertion resulting in hyperthermia and/or myopathy

Truck: When transporting Black rhinos by truck, open trailers should be protected from excessive wind, rain and sun. In extreme hot or cold temperatures an enclosed trailer is an option. In any case, the vehicle must be climate controlled.

Airplane: The International Air Transport Association (IATA) has made the IATA Live Animals Regulations (LAR). These Live Animal Regulations are a Worldwide Standards for transporting live animals by airlines. The objective of the IATA Live Animals Regulations is to ensure that all animals are transported safely and humanely by air, whether it is to transport a pet, an animal for zoological or agricultural purposes or for any other reason *(IATA, 2007).*

The IATA Live Animals Regulations are applicable to members of the International Air Transport Association according to the provisions of Cargo Services Conference Resolution 620 and to airlines being parties to the IATA Multilateral Interline Traffic Agreement-Cargo *(IATA, 2006).*

The IATA Live Animals Regulations are accepted by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and the Office International de Epizooties (OIE) as guidelines in respect to transportation of animals by air. These regulations have been used by the Council of Europe as a basis for its code of conduct for the international transport of farm animals. The European Union has adopted the IATA Live Animals Regulations as the minimum standard for transporting animals in containers, pens and stalls. As an increasing number of countries adopted or accepted these regulations as a part of their national legislation, shippers are warned that shipping live animals in violation of the regulations may constitute a breach of the applicable law and may be subject to legal penalties.

The IATA Live Animals Regulations container requirement 71 concerns the rhino species.

Transport box / crate: The IATA Live Animals Regulations container requirement 71 states the requirements of the transport container that is applicable to transport of Elephants, Hippopotamus and Rhino species (IATA, 2006).

Material and dimensions: Materials that can be used to construct a transport container, according to the container requirement 71, are metal and hardwood. The transport container should be big enough to restrict the movement as well as restrain the animal in question. The animal must be able to stand naturally without being cramped but must not be able to move freely (IATA, 2006).

Container dimensions should be determined by the animal's size. In general, the container should be 30 cm longer and wider than the animal when it is lying on its side. Approximate container dimensions are 350 cm in length, a height of 191 cm height, and 140 cm wide to prevent the animal from turning around (Fouraker and Wagener, 1996).

Frame and slides: The frame of the transport container should be made out of strong metal welded or bolted together depending on the weight of the animal. Solid hardwood sides, with no internal projections, must line the outer framework for extra strength. All woodwork must be secured with bolts and nuts that face the exterior so that they can be easily tightened from the outside. Spring steel weld mesh can also be used in combination with strong metal corner posts, together with a rigidly braced top and sides. In either case the lower part of the sides must be solid and leak-proof. A heavy plastic foil or tarpaulin covered with sufficient absorbent material which is tied up half a meter around the crate can be used (IATA, 2006).

Floor: The floor must be made of thick tongue and groove of at least 2.5cm (1in.) thickness or its equivalent and have a non-slip surface. It must be completely leak-proof (IATA, 2006).

Roof: The roof must be solid over the animal's head and shoulders and slatted over the loins and hindquarters to give good ventilation (IATA, 2006).

Doors: A series of metal bars must be bolted to the top and bottom of both the entry and exit of the container. Exterior to these bars sliding or hinged solid hardwood entry and exit doors must be made to completely cover the entry and exit. The doors must be fastened by a sufficient number of strong bolts which must be able to resist the weight of the animal. The upper third of both doors must have ventilation openings. Entry and exit must be clearly marked as such (IATA, 2006).

Ventilation: Through the slatted or louvered upper third of both wooden doors and the slatted portion of the roof there should be adequate ventilation (IATA, 2006).

Feed and water containers: The water container must be fixed in the front of the container. It must be made of strong metal and wide enough for the animal's muzzle to enter. The edges of the water trough should be smooth so the animal cannot hurt itself. For feeding, outside access can be from a low wooden flap, clearly marked "Feeding" at the base of the door. Food can be placed between the bars and the door. The access flap must be securely closed when not in use (IATA, 2006).

Forklift extrusions: Forklift extrusions must be provided as an integral part of the design (IATA, 2006). An example of a container that can be used for rhinos is shown in Figure 3.8.

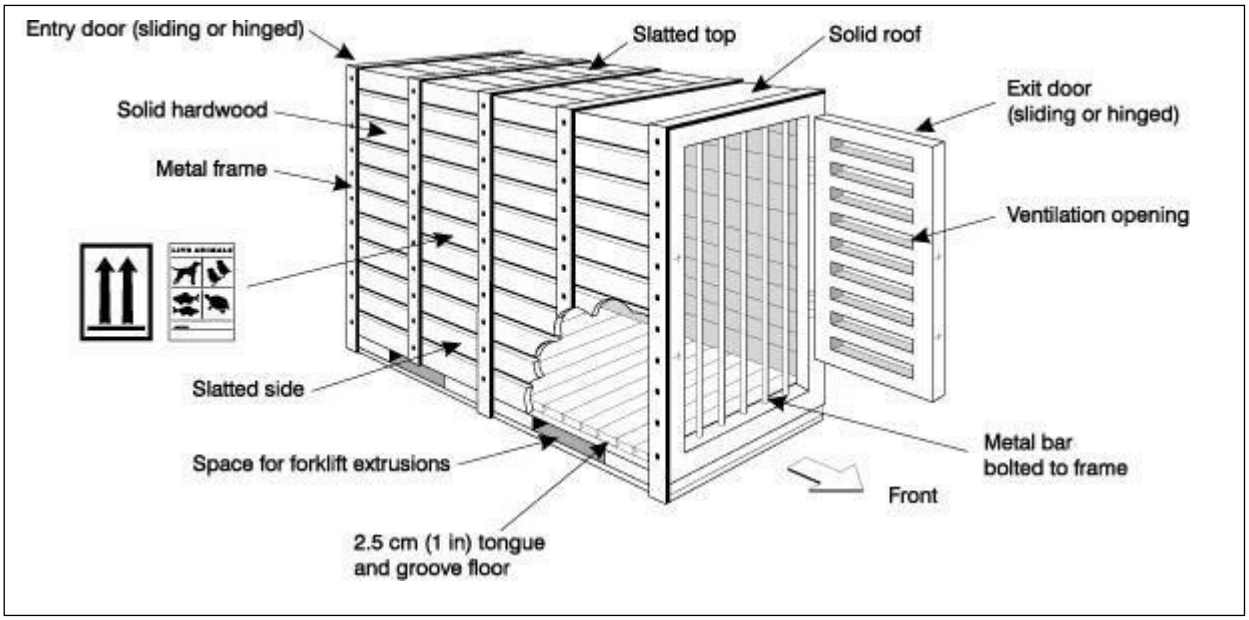


Figure 3.8: Example transport container for rhinos (IATA, 2006).

An ample amount of absorbent material such as wood shavings is required for bedding. The animal must be watered before shipment. Animals do not normally require additional feeding or watering during 24 hours following the time of dispatch. If feeding or watering is required due to an unforeseen delay, instructions supplied by the shipper must be followed (IATA, 2006).

It is recommended that all shipments of these species be accompanied by a person/veterinary and go through a crating training well before dispatch (IATA, 2006). We refer to section *2.6 Behavioural enrichment* for more information about crate training. A light sedation is recommended for transports taking longer than a couple of hours (Fouraker and Wagener, 1996).

Markings on transport container: The markings on the transport container must be durable and printed or otherwise marked on or affixed to the external surface of the live animal container. English must be used in addition to the language which may be required by the state of origin (IATA, 2006).

Unless otherwise specified in these Regulations, each live animal container must be marked, durably and legibly on the outside of the container, with each of the following:

- The full name and address and contact number of the shipper, consignee and a 24-hour contact (if it is not one of the aforementioned persons responsible for the shipment).
- The scientific and common name of the animal(s) and quantity of each animal contained in the container, as shown on the shipper's certification.
- Containers carrying animals which can inflict poisonous bites or stings must be boldly marked "POISONOUS". Aggressive animals or birds that can possibly inflict injury through the bars or ventilation openings of the container must have an additional warning label "This Animal Bites".

- Affix special feeding and watering instructions to the container.
- In general, tranquillisation is not advocated for the transportation of live animals. However, certain wild species require the use of such medication. Whenever used, they must be administered under competent supervision and the name of the sedative, time of administration and the route of administration must be clearly marked on the container and a copy of the record must be attached to the documents relating to that shipment. Any further medication administered must be recorded and accompany the shipment with the name of the sedative, time of administration and the route of administration (IATA, 2006).

It is mandatory to attach at least one IATA "Live Animals" or one "Laboratory Animals" label or tag, properly completed, to each live animal container, unless otherwise stated in the individual container requirements. Animal containers may have the appropriate labelling imprinted (IATA, 2006). The label for live animals should have the following header "Live Animals", the colour should be bright green on a light background. The minimum dimensions of the label are 10 cm x 15 cm and letters of 2.5 cm (IATA, 2006). In Figure 3.9 the label for live animals is shown.

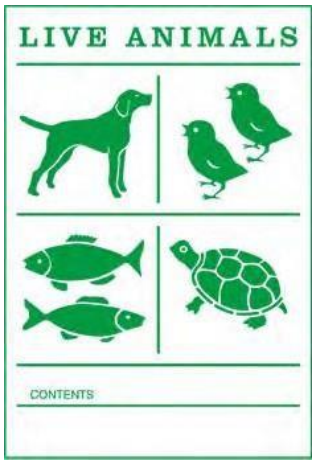


Figure 3.9: Live animals label (IATA, 2006).

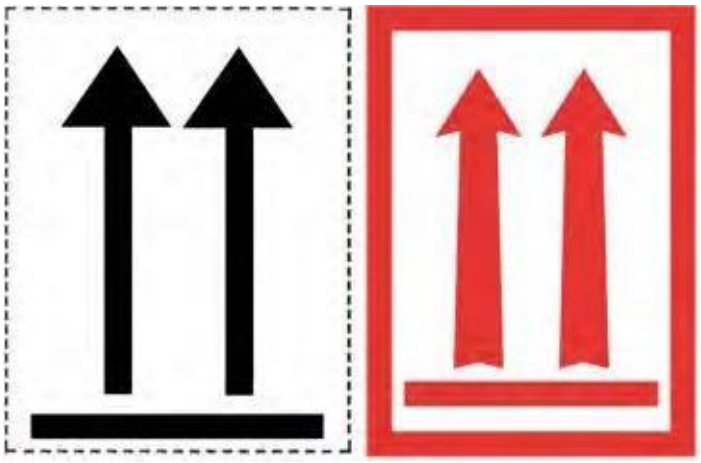


Figure 4.0: This way up label (IATA, 2006).

In addition to the "Live Animals" label, it is mandatory that the "This Way Up" labels or markings be placed on at least two opposite sides. Labels may be imprinted on the container. The label for "This way up" should be black or red on a contrasting background. The minimum dimensions of the label are 74 mm x 105 mm and letters (IATA, 2006). In Figure 4.0 the label for "This way up" is shown.

2.7.5 Safety

In order to ensure safety and to properly meet the requirements of management it is recommended that more than one keeper be responsible for these animals on a daily basis. Keeper interaction should be restricted to designated areas and should be conducted in accordance with institutional protocols. Consistency of routine is vital in daily interaction (Fouraker and Wagener, 1996). Keepers should always carry a radio and / or a mobile phone when working with rhinos.

2.7.6 Stress

Moving animals between institutions is an important aspect of population management, but could also be considered as a potential stressor, both in terms of the transport involved and the novelsocial and physical environment into which the rhino is moved. Hormone analysis can also be used asa tool to investigate the role of potential stressors on adrenal activity, by measuring the steroid hormones glucocorticoids. The adrenal response to a stressor in the first instance is not a bad thing, but demonstrates that the animal is responding accordingly to a potential threat. However, if stressors are particularly severe, or are prolonged, then negative consequences on health and reproduction could result.

To investigate short-term or long-term effects of translocation, hormone analysis was conducted following the translocation of four male and five female black rhinos between European institutions between 2008 and 2012. Although an adrenal response to translocation was observed in some individuals following inter-zoo transfer, this was not apparent in all individuals. Furthermore, following these translocations, there was no evidence of oestrous cycle disruption; three out of five females were sexually mature and oestrous cycles continued post-translocation, one has since produced a calf. The remaining two females were not yet cycling prior to translocation, but one of those since commenced regular cyclicity and has now produced a calf. In males, no consistentdifferences in testosterone concentration were observed post-transfer, and one male sired a calf approximately twelve months post-transfer.

Behavioural indicators for stress are animals with head up and looking around or running with tail up, pacing during the night; which is evident by the night bedding being spread around the night area, abnormal amount of moving and pacing and aggression against fence, person etc.

There are several causes for stress in Black rhinos and these should be avoided:

- inability to escape entirely from other Black rhinos
- boredom
- inability to display all of their natural behaviour patterns
- stressed by their environment by public, machinery, etc.
- lack of visual barriers
- permanent separation of mother and calf before age of 18 months

Stereotypical behaviour mentioned by the experts are walking back and forth, caused by not enough hiding possibilities and running on one path only, it is not known what causes this behaviour.

2.8 Veterinary: Considerations for health and welfare

The following Black Rhinoceros (*Diceros bicornis)* Veterinary Guidelines have been created by:

Jane Hopper, Aspinall Foundation, Uk, Javier Lopez, Chester Zoo, UK, Linda van Sonsbeek, Rotterdam Zoo, NL, Julia Stagegaard, Ree Park Safari, DK, Robert Hermes, IZW, GER,Marcus Clauss, University of Zurich, SW.

In captivity black rhino occasionally display unusual disease syndromes not described in the wild (Dennis, 2007). Black rhino appear to be more susceptible to a variety of diseases that are still not fully understood (Fowler and Miller, 2003).

Husbandry procedures that facilitate access to diagnostic samples, especially blood, are therefore more important in the black rhino than in many other species, as progress in understanding black rhinoceros health will depend critically on access to samples.

Regarding illness, the behavioural repertoire of rhino is often quite limited. Depression, inappetence and loss of body condition are often the only signs of major disease problems (Fouraker, 1996). In this chapter, basics of medical procedures, diseases and disorders concerning the black rhino are described.

2.8.1 Medical procedures

Physical exam

It is often possible to get some basic data from a black rhino without sedation. Additionally, training should be performed to allow blood sample collection and further examination.

Clinical parameter	Black rhino reference range
Resting heart rate	30-40 bpm in an adult
Resting respiratory rate	8-12/ min in an adult
Rectal temperature (awake animal)	36-37.5°C in adults
Body weight	Adult females 800-1200kg. Adult males 1000-1350kg. Neonates 40-50kg

(Fowler and Miller 2003; *pers comm* Pete Morkel)

Blood collection

With training or restraint devices, blood can often be collected from an awake black rhino. This should only be carried out when safe to do so, and when the appropriate risk assessments have been

carried out. The most commonly used sites are the radial vein, metacarpal vein and auricular vein. This is most easily achieved using a butterfly catheter. The coccygeal vein can also be used. It can be hard to collect a large sample from the auricular vein. Large volumes of blood can be collected from the radial or metacarpal veins for diagnostic testing, therapeutic phlebotomy or plasma/ serum banking (Mylniczenko, 2012). Up to 8 litres of blood has been collected from these sites. Recently a technique has been published for venipuncture of the transverse facial vein in black rhino. This allowed large volumes of blood to be collected (Schlanser, 2016).

Arterial samples for blood gas analysis are most easily collected through using the medial auricular artery (inside the ear).

Haematology, biochemistry, mineral, protein electrophoresis and blood gas values are listed below (ZIMS 2018).

Haematology parameter	Black rhino reference range
RBC ($x10^{12}$/µl)	2.41-5.86
Haemoglobin (g/dl)	8.4-16.4
Haematocrit (%)	25-45
MCV (fl)	71.1-103.0
MCH (pg)	24.4-36.7
MCHC (g/dl)	30.0-39.7
Nucleated RBC (/100 WBC)	0-2
Platelets ($x10^{12}$/l)	0.055-0.487
WBC ($x10^{9}$/l)	4.2-12.7
Lymphocytes ($x10^{9}$/l)	0.58-3.80
Monocytes ($x10^{9}$/l)	0.00-0.946
Segmented neutrophils ($x10^{9}$/l)	0.01-8.34
Band neutrophils ($x10^{9}$/l)	0.033-0.535
Eosinophils ($x10^{9}$/l)	0.000-0.765
Basophils ($x10^{9}$/l)	0.000-0.282

Serum mineral/ blood gas parameter	Black rhino reference range
Sodium (mmol/l)	122-139
Potassium (mmol/l)	2.9-5.8
Chloride (mmol/l)	89-103
Calcium (mmol/l)	2.7-3.6
Phosphorus (mmol/l)	0.52-2.11
Ionised calcium (mmol/l)	0.35-1.80
Magnesium (mmol/l)	0.45-1.40
Bicarbonate (mmol/l)	15.5-29.7
CO_2 partial pressure (mmHg)	25-83

Biochemical parameter	Black rhino reference range
Glucose (mmol/l)	1.2- 6.4
Urea (mmol/l)	2.5-8.2
Creatinine (µmol/l)	51-133
ALT (U/l)	3-46
AST (U/l)	41-165
LDH (U/L)	192-992
ALP (U/L)	12-167
Gamma GT (U/l)	11-72
Amylase (U/l)	0-36
Lipase (U/l)	0-27
CK (U/l)	152-744
T-bil (µmol/l)	1.7-10.3
TP (g/l)	61-94
Albumin (g/l)	13-33
Globulin (g/l)	44-78
Fibrinogen (g/l)	0.00-5.83

All haematology, mineral, blood gas and biochemical parameters from ZIMS (both sexes combined and all ages combined).

Biochemical parameter relating to iron status (see chapter Diseases of unknown origin, Iron Overload Disorder)	Black rhino measurements
Iron (µmol/l)	21.2-66.2
Ferritin (pmol/l)[1]	261-9715
TIBC or Total Iron Binding Capacity (µmol/l)[2]	90-105.1[4]
TS or Transferrin Saturation (%)[3]	27-70[4]

[1]analysis currently not available in Europe; value range includes magnitudes considered not normal
[2]necessary for TS calculation; not offered by many labs
[3]calculated from serum iron and TIBC; note that lab methods measuring 'transferrin' for human diagnostics do not correspond to this measure
[4]Values from ZIMS, adjusted based on experience Rotterdam Zoo

Urine collection

It is relatively easy to collect a free catch urine sample from a rhino using a sterile pot attached to a stick of an appropriate length. This should only be carried out when safe to do so, and when the appropriate risk assessments have been carried out. Rhino urine physiologically contains large numbers of calcium carbonate crystals which makes it have a milky appearance. Calcium oxalate, phosphate and ammonium crystals may also be seen depending on the diet. Certain browse species (e.g. ash, mulberry, evergreen oak) may cause the urine to become very dark in colour. Analysis

should be performed to rule out any abnormalities. Published normal values for adults are listed below (Haffey, 2008).

Parameter	Black rhino reference range
pH	8.10-8.26
SG	1.010-1.012

Urinary catheters are extremely difficult to place in females as the cervical canal is long and tortuous and characterised by interdigitated folds. It has been achieved in black rhino under ultrasonic guidance (Fowler and Miller, 2003).

Cerebrospinal fluid collection

Due to the large size of the black rhino cerebrospinal fluid (CSF) has only been successfully collected from rhino calves. Lumbar or cisternal taps have been used (Miller, 2003).

Diagnostic Imaging

Radiography in adult black rhino is limited to the distal extremities, skull and horn. Radiography can be used more widely in calves. Examination of the female urogenital organs (vagina, cervix, uterus ovaries, urethra, bladder, kidneys) in adult rhino may be attempted with standard ultrasound units. Black rhino brains have been imaged using magnetic resonance imaging (Bhagwandin, 2017).

Thermography

Thermography is widely used in rhino in many institutions to localise areas of inflammation associated with lameness. Thermography can also be used to detect changes in skin temperatures with skin disease (Hilsberg-Merz, 2008).

Surgery

Most surgical procedures involve the skin, eyes, digits and horn. Sutured rhino skin wounds often dehisce due to the skin's thickness and inelasticity. The thick skin of rhino does not lend itself to primary closure. Therefore, wounds are usually left to heal by secondary intention using analgesia, antibiotics (chosen according to culture and sensitivity), topical treatments and debridement. Vacuum assisted healing has been used for the surgical wound of a black rhino and reduced healing time (Harrison, 2011).

Surgical treatment of osteomyelitis in a black rhino has been described (Harrison, 2011). Surgical repair of a black rhino with a rectal prolapse has also been reported, although it was ultimately unsuccessful (Olivier et al, 2001).

Laparoscopic techniques have been used for reproductive procedures (Hermes, 2012).

Intravenous fluids

Due to the size of adult black rhino it is difficult to provide enough intravenous fluids for long-term support. However, hypertonic saline can be used in emergency situations to support the circulation. Black rhino calves can be given intravenous fluids more easily and can tolerate the fluid lines and cannula well. Adult rhino often pull out the IV line, either when moving around or due to intolerance of the line. In both adults and calves the easiest place to place intravenous cannulae are the ear veins as the skin over these veins is thinner than elsewhere on the body.

Hydration can be improved in adult animals by using rectal enemas (approx. 30 litres) of warmed physiologic solutions or even warmed tap water using a tube and stirrup pump.

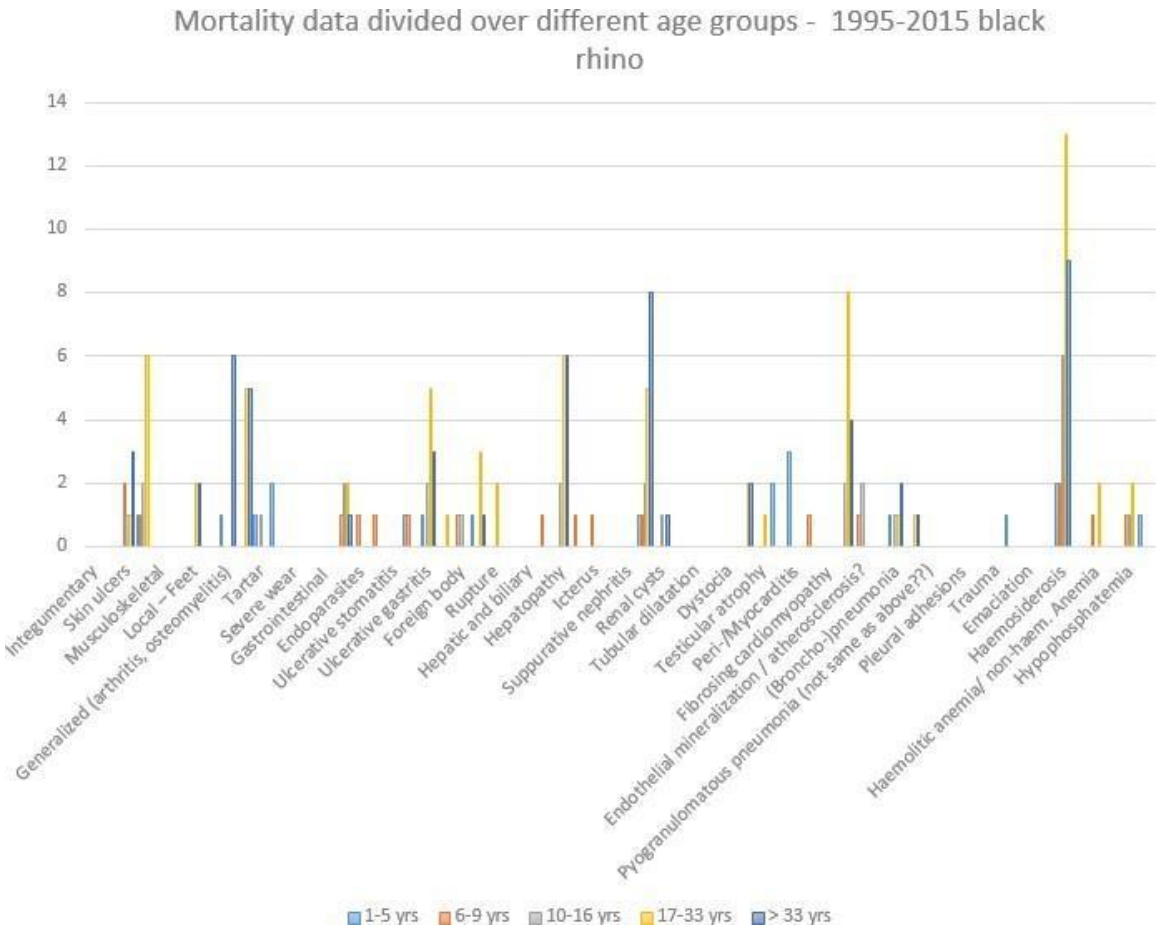
Neonatal examinations

Neonatal examinations can be performed, although it is often impossible to do so. The dam must be separated for these exams. The exam should include weight, full examination (including umbilical examination), and microchip placement. Tests can be carried out to determine if passive transfer of immunoglobulins has occurred (glutaraldehyde coagulation, zinc sulphate turbidity, radial immunodiffusion plates and serum protein electrophoresis).

Black rhino calves have been recorded with congenital deformities. One calf had a cleft palate, patent foramen ovale and 4 septal defects (Lewis, 2016) and another had cardiac truncus arteriosus (Rodriguez, 2017).

2.8.2 Disease

Summary necropsy data 1995-2015



Infectious diseases

Tuberculosis

Infections with both *Mycobacterium tuberculosis* and *Mycobacterium bovis* have been reported in captive rhino (Espie, 2009; Miller, 2015) and *M. bovis* has been reported in a free ranging black rhino (Miller, 2016). However, the total number of 22 incidental tb cases across all rhinoceros species reported so far remains low compared to other ungulates such as tapirs, or in elephants.

Initial infections may be asymptomatic or result in progressive weight loss. In all cases of tuberculosis in rhino, the pulmonary system was the primary infected area. In these cases, nasal discharge, coughing and dyspnoea may be seen before death.

Pre-mortem testing may be attempted with intradermal tuberculin in the eyelid, tail fold, or the skin at the base of the ear or pinna. Comparative testing is usually carried out in the tail fold and repeated ten days later in the neck if a suspect reaction occurs in the tail is recommended (Fowler, 2003). Interpretation of (CITTT): Considering the large numbers of antigens included in PPD tuberculins, and given the high exposure of rhinoceros to environmental mycobacterial antigens increasing the risk of

cross reactive bovine PPD skin reactions, only the comparative test (CITT) should be used. Skin test results can be very inconsistent, and the predictive values are suspected to be low; thus, even CITT results should be interpreted cautiously, and are more often indicative of any mycobacterial exposure (i.e., to nontuberculous mycobacteria) when positive.

Cave: when using an intradermal tuberculin test the animal's immune system is primed against large variety of Tb antigens and likely to produce false positive results in serology test showing false positive reactions based on the tuberculin testing.

Tracheal lavage, gastric lavage or both can also be performed for PCR testing and mycobacterial culture. The ElephantTB STAT-PAK Assay (CHEMBIO Diagnostic Systems, Medford NY, USA) antibody test kit has given positive results when used retrospectively on sera of rhino infected with tuberculosis (Duncan, 2009) and the dual path platform test, gamma interferon testing and multi- antigen print immunoassays have been used in the recorded black rhino cases of mycobacterium. Please see Appendix 4 for further details of testing rhino for *Mycobacterium*. One case of *M. tuberculosis* in a captive black rhino was found to have tested positive for the STAT PAK, dual path platform and multi-antigen print immunoassay for over 12 years prior to death (Miller, 2015). A blackrhino that had recently been brought into captivity was found to have *M. bovis* at post-mortem examination (Espie, 2009). EAZA guidelines on TB testing in rhino are included in Appendix 4.

Treatment has been attempted with isoniazid, rifampin, ethambutol and pyrazidamide but successful responses have been limited. Further treatment details can be found in Appendix 4. Prior to starting treatment, the health risks for staff members and other collection animals must be considered.
Mycobacterium avium paratuberculosis (Johnes disease) has been isolated from a wild caught black rhino (Bryant et al 2012). The rhino had diarrhoea. Clinical signs resolved and faecal culture became negative after a course of antimycobacterial drugs.

Salmonella

Salmonella has been reported to cause both enteritis and sepsis in black rhino. Research has shown that asymptomatic black rhino may intermittently carry and shed Salmonella in their faeces. Asymptomatic rhinos should not be treated. A retrospective survey of captive black, white and greater Asian one-horned rhino in the USA reported that 11% of rhino in the study had positive cultures for salmonella, usually associated with clinical signs (Kenney, 1999). Another survey showed that testing for salmonella using multiple diagnostic methods and increasing the number of samples analysed increases the likelihood of identifying black rhino that are asymptomatic shedders of salmonella.

Common symptoms of salmonella infection include anorexia, diarrhoea, abdominal pain and lethargy. Treatment should be with an appropriate antibiotic based on culture and sensitivity. Salmonella has been described in black rhino calves (Love, 2017). Presentation and outcome were variable with two fatal cases and two calves making a full recovery post treatment.

Leptospirosis

Leptospirosis has been associated with some cases of primary haemolytic anaemia. However, it usually presents as depression and anorexia. Haematology may show a haemolytic anaemia. Haemoglobinuria, colic and skin ulcers may also be present. Diagnosis is based on high antibody titres to leptospira and the detection of leptospiral organisms in urine or tissues. Fatality rates in black rhino with leptospirosis are high, although successful treatment with TMPS and ceftiofur have been reported (Neiffer, 2001). Low levels of antibodies have been observed in non-vaccinated free ranging black rhino without evidence of disease. Black rhino can be vaccinated annually with a multivalent large animal vaccine. However, vaccination against leptospirosis is not widely or routinelypracticed within EAZA institutions. Rodent control and good husbandry to minimise contamination offood and water by rodents are also important to limit the spread of this disease.

Encephalomyocarditis infection

Acute death following infection with encephalomyocarditis virus has been noted in black rhino(Gaskin, 1980). Diagnosis is usually at post-mortem and is based on virus isolation from the heart or other tissues. Rodent control is key to preventing this disease.

Fungal pneumonia

Fungal pneumonia had been reported in several black rhino, primarily secondary to immunocompromise from concurrent disease, broad-spectrum antibiotic use or corticosteroid use (Weber, 1996). Nearly all the cases have involved infection with Aspergillus spp., and several of these have followed corticosteroid therapy, sometimes even relatively low doses administered over short treatment periods. Fungal pneumonia should be considered in all black rhino with signs of respiratory illness. Symptoms are those typical of pneumonia including weakness, weight loss and epistaxis. Diagnosis can be difficult but bronchoscopy with fungal culture and cytology as well as serology can be performed. The efficacy of long-term anti-fungal drugs is unknown. Fungal pneumonia is often associated with very high serum globulin levels, higher than those generally observed in chronic diseases (Citino, *pers comm.).*

Clostridial disease

Clostridial disease is not common in black rhino. Clostridial enterocolitis has been observed in black rhino with signs of diarrhoea, lethargy and colic. Fatal cases of enterotoxaemia due to clostridial enteritis have occurred in black rhino (Ndeereh, 2012). A case of tetanus and a case of *Clostridium sordelli* have also been recorded (Fouraker, 1996). Animals considered to be at risk (e.g. when conspecifics have been affected) can be vaccinated.

Parasites

Parasites are usually only found in low numbers in captive black rhino. These low burdens are usually not associated with clinical signs. In newly captured rhino, consideration should be given to blood and skin parasites as well as gastrointestinal parasites. A biannual faecal examination for parasites is adequate in rhino established in captivity. In newly captive rhino blood examinations should be performed for blood parasites (e.g. Babesia sp., trypanosomes, theileriasis and leishmaniasis). Skin lesions in wild caught black rhino should be biopsied and examined for the presence of *Stephanofilaria dinniki.* The most commonly found endoparasites in newly captive rhino have been tapeworms which can cause asymptomatic infestation in both wild and captive rhino (Miller, 2003). Stress (transportation, translocation, etc.) may exacerbate parasitic infestations, increasing the risk of clinical signs and the introduction of novel pathogens to a new environment. Rhino should also be screened for parasites pre-transfer and treated if positive (see formulary).

Poxvirus

Poxviral infection has been recorded in a black rhinoceros (Eulenberger, 2005). The rhinoceros presented with pyrexia, conjunctivitis, otitis and a severe swelling of the entire neck region. Typical pox erosions were initially found on the buccal mucosa and these then spread and were found all over the skin and the genital region. Two weeks post onset pox lesions were seen at the coronary band. A diagnosis of cowpox virus was made by electron microscopy of skin biopsies. Cowpox virus was also isolated from the scabs. Treatment consisted of antibiotics for secondary infection, the antiviral drug famciclovir, probiotics and high doses of vitamins. The lesions were treated topically with iodine. Other rhinoceros in the collection were successfully vaccinated against cowpox virus.

Non infectious diseases

Skin wounds

Traumatic injuries in black rhino are relatively common. Injuries are most commonly due to fighting (conspecific or interspecific), mating injuries and injuries from enclosures. The thick skin of rhinodoes not lend itself to primary closure. Therefore, wounds are usually left to heal by secondary intention using analgesia, antibiotics (chosen according to culture and sensitivity), topical treatments and debridement. Vacuum assisted healing has been used for the surgical wound of a black rhino and reduced healing time (Harrison, 2011).

Horn damage, trauma or avulsion

These horn conditions can occur due to fighting, chronic rubbing or damage from the enclosure. The use of horizontal bars under which rhino can hook their horns should be avoided. Horn damage can

be assessed with radiography, thermography and fluoroscopy. Treatment can include debridement, antibiotics, fly control and topical therapies. When a horn is avulsed there can be notable haemorrhage from the base of the horn, but these usually stop without intervention.

As the horn is attached to the basal dermal layer, disruptions in blood flow may lead to laminitis (see further information in 'Foot problems' section below).

Tartar accumulation

Black rhino readily accumulate dental tartar in captivity, especially if they do not have access to enough browse. It is assumed that longer fibre lengths in the diet lead to longer mastication times and therefore less tartar build up. Some black rhino develop proliferative gingivitis disproportionate to the amount of calculus present (Beagley, 2010). In many cases dental disease in rhino has only been noticed at post-mortem examination. How well rhino masticate their food can be assessed by analysing the fibre lengths in their faeces, and this is a useful way of assessing dental health. Unfortunately there is as yet no data available on the expected fibre length in rhino faeces.

Neoplasia

Neoplasia is rarely reported in black rhino. Cases of squamous cell carcinoma and melanoma have been recorded (Wack, 2010). Thyroid carcinoma, hepatic carcinoma, seminoma (Portas, 2010) and acute lymphoblastic leukaemia (Radcliffe, 2000) have also been recorded.

Gastrointestinal disease

Gastrointestinal torsion and impaction have been reported in black rhino with signs similar to those of colic in the horse.

Torsion may result from abdominal trauma or severe gastrointestinal disease. Clinical signs with torsion range abdominal pain to acute death. Torsions should be surgically corrected if possible. Impaction colic can result from the provision of poor quality forage, sand ingestion, dehydration, inadequate fibre provision or from dental disease. This highlights the importance of providing black rhino good quality forage, a suitable balanced diet and keeping them on appropriate substrates (see husbandry section). Treatment is similar to that in the horse, with rectal enemas and oral psyllium, mineral oil or other products that increase gastrointestinal water content.
Gastric ulcers are commonly seen at the post-mortem examination of rhino that have received long-term non-steroidal anti-inflammatory treatment or those with concurrent disease. For this reason, black rhino on long-term courses of non-steroidal anti inflammatories should be prescribed a gastroprotectant such as omeprazole.

Choke (oesophageal obstruction) has been observed in black rhino with dental disease. An endoscope can be passed through the nasal meatus and the blockage visualised in the oesophagus.

Oesophageal dilatation was seen in a black rhino following ingestion of a foreign body. This was successfully managed by feeding a low fibre diet. Diagnosis was by endoscopy (Radcliffe, 1998).

Recurrent regurgitation of milk has been seen in a black rhino calf with inflammation of the distal oesophagus near the pylorus. This was diagnosed with endoscopy and successfully managed with oral sucralfate.

Failure to pass faeces has been seen in black rhino calves who have eaten items inappropriate for their age (browse, mud, lucerne etc.). However, it can be normal for calves not to pass faeces until they are ten days of age. This can be successfully managed with large volume enemas of warmed lubricant given rectally using a stomach tube attached to a stirrup pump. Volumes vary with age but 10 litres has been used successfully in a two month old calf. For this reason it is prudent to feed the mothers at a height or in a way that calves cannot access the food until they are old enough to do so. Rectal prolapse has been reported in black rhino calves, with surgical intervention needed in at least two cases (Pearson, 1967; Abou-Madi, 1996).

Renal disease

Chronic glomerulonephritis and/or renal failure has been found to contribute to cause of death in several black rhino since 2001. Clinical signs can include weight loss, decreased appetite, dermatitis and signs of gastric ulcers. Significant azotaemia and changes in urinalysis were not present in all these cases, making a diagnosis difficult until post-mortem examination. Mineralisation of other tissues has been associated with chronic renal changes (Murnane, 1994).

Reproductive diseases

The reproductive cycle in female rhino is monitored by hormone analysis of serum or faecal samples. In females without a regular or any oestrus cycle activity oral hormone treatment can induce oestrous and ovulation with subsequent mating. Such oestrous inductions have repeatedly led to pregnancies (Schwarz, 2014).

Female black rhino may acquire a range of non-infectious, reproductive disorders over time. These include endometrial cystic degeneration, uterine leiomyoma, adenoma or adenocarcinoma, oviductal cysts and ovarian cysts. The lesions are mostly asymptomatic and might be disguised by a normal oestrous behaviour and regular endocrine profile. However, they might interfere greatly with reproductive success. One single ultrasound examination may determine the presence of an existing reproductive disorder and remaining breeding potential of the rhino.

Reproductive disorders in male rhino are rare. However, males may develop testicular tumours (Portas, 2010). Testicular seminoma, in equids potentially malignant, require hemicastration of the affected testis. Spermatogenesis might resume after such hemicastration.

Foot problems

Rhino are susceptible to developing pododermatitis due to inappropriate substrate (e.g. abrasive substrate), the build-up of urine and faeces in enclosures, limited access to wallows, or waterlogged paddocks. Treatment should aim to improve husbandry, regular foot trimming and surgical debridement.

Black rhino have reportedly developed laminitis that may be related to IHVS (see diseases of unknown aetiology) (Nance, 1998). This condition can be managed with frequent foot trimming, analgesia and antibiotics.

Stress

Black rhino seem very susceptible to stress and benefit from some parts of their enclosures being off show to the public. Transportation is another obvious time of stress, and tranquilisers are recommended (see section on crating). Stress has been associated with several diseases in black rhino. Whenever a black rhino becomes unwell, it is prudent to re-assess its environment, diet, social grouping, etc., scrutinizing changes of routines, conditions or the environment. Mild episodes of skin disease, cases of slight weight loss, lethargy and depression have commonly been observed to be associated with slightly suboptimal housing temperatures, forage quality or the presence or absence of other black rhino.

Toxins

Seven deaths occurred in a group of 20 black rhino in Zimbabwe housed in bomas constructed with creosote treated wood (Kock, 1994). Clinical signs seen included lethargy, reduced appetite, swollen limbs and brown urine. Cause of death seemed to be liver dysfunction due to creosote toxicosis. Exposure to creosote treated housing materials should be avoided for all rhino.

Chronic renal changes and mineralisation of other tissues were seen in three black rhino in a case of accidental vitamin D toxicosis (Fleming, 2003).

Vitiligo

Vitiligo has been recorded in a two-year-old captive female black rhino (Takle, 2010) that developed symmetrical multifocal depigmented areas. Treatment with UV-B resulted in significant repigmentation.

Diseases of unknown aetiology

Superficial necrolytic dermatopathy (SND)

SND presents as small skin plaques or vesicles that progress to ulcers (Munson, 1999; Dennis, 2007). These lesions are most commonly seen over the pressure points but they can appear anywhere including in the mouth and on the nasal mucosa. Affected rhino can be asymptomatic or can be depressed, anorexic and lame, have oral or nasal bleeding and lose weight. Oral or nasal bleeding can be seen if the lesions affect these areas. Affected animals may have hypoalbuminaemia and low haematocrit. Treatment and management is symptomatic although steroids and cryosurgery has been successful in some cases. The outcome of SND is very variable- the lesions can resolve, become waxing and waning or the condition may be fatal. A specific cause of SND has not been found, but most rhino have other concurrent health problems or problems with stress. Research suggests the underlying cause may be an imbalance of dietary essential micronutrients with related metabolic changes. One study found that hypoaminoacidaemia was not associated with ulcerative lesions in black rhino (Dorsey, 2010). Another study found that ulcerative lesions may be associated with changes in adrenal activity, although it was not clear whether this is the cause or effect of the ulceration (Dorsey, 2010).

Idiopathic haemorrhagic vasculopathy syndrome (IHVS)

The initial signs of IHVS are usually severe limb, facial and neck swelling associated with non-haemolytic anaemia (Murray, 2000). Lethargy, respiratory stridor, laminitis, nail sloughing, ulcers and aural haematomas can also be seen. The progression of the cases is usually acute death, often occurring within 48 hours of the initial signs. The fatality rate of IHVS is high but a number of animals have recovered with antibiotic and non-steroidal therapy, although recurrent episodes are likely. Treatment can also include fatty acid and phosphorus supplementation. The syndrome may be an immune-mediated vasculitis (Murray, 2000).

Haemolytic anaemia

The prevalence of haemolytic anaemia in black rhino has decreased a lot since the 1990s. Recorded cases have a fatality rate of 75% (Miller, 1993: Dennis, 2007). Management of the anaemia includes phosphorus supplementation, vitamin E, prophylactic antibiotics and whole blood transfusion. The definitive cause has not been determined. Leptospirosis was associated with some cases and other possible aetiologies include hypophosphataemia, hypovitaminosis E, hereditary deficiency of glucose-6-phosphate dehydrogenase. Research has suggested that the red blood cell of the black rhino is inherently energy deficient and thus unstable and susceptible to haemolysis (Fouraker, 1996).

Hypophosphataemia

Hypophosphataemia is a recognised problem in captive black rhino with levels dropping below1mg/dl. Low serum phosphorus has been linked to haemolytic anaemia and other blood disorders (Miller, 1993; Dennis, 2007). Oral and intravenous supplementation can be instituted in these cases. In critical cases, potassium phosphate can be administered at 14.5mmol P/hour. Serum calcium should be carefully monitored.

Eosinophilic granuloma syndrome

This syndrome usually presents with a non-healing ulcerated mass that either bleeds profusely(Pessier, 2004) or appears jellified. Common sites include the mouth and nasal mucosa although they have also been recorded on the feet and nail cuticle. Cytology from these lesions shows a predominance of eosinophils. Although lesions may resolve spontaneously, usually over several months, they may recur. Treatment options include oral steroids with anti-histamines or cryosurgery.Rapid regrowth of the mass is common after cryosurgery. Oral steroids usually cause the disappearance of the granuloma, and oral anti-histamines appear to decrease the frequency ofappearance of new granulomata (Bishop, 2016). Laser therapy has been used to treat granulomas but may exacerbate haemorrhage. Eosinophilic granulomas in wild rhino are typically associated with *Stephanofilaria dinniki*.

Leukoencephalomalacia

Leukoencephalomalacia has occurred in young black rhino. It is caused by necrosis of the cerebrum. In three of the four cases, it presented as acute and profound stupor. These calves became comatose and eventually died. The fourth case experienced 14 episodes over a number of months before being euthanized. The aetiology may be related to dam age or excessive maternal iron. These cases emphasise the importance of collecting brain and central nervous system tissue on all rhino necropsies (Fouraker, 1996).

Iron Overload disorder (IOD)

Husbandry philosophy: IOD of black rhinos is a peculiar topic, because the term describes, on the one hand, a reality reflected in serum biochemistry and post mortem examinations, but on the other hand, its etiopathology remains completely obscure today. In the absence of a known etiopathology, the only logical ways to address the problem are, on the one hand, reasonable preventative measures – feeding of diets with low iron levels, and phlebotomy to reduce iron loads in live animals with careful monitoring, and on the other hand continuous efforts to investigate the problem – for which regular access to blood samples of live animals is a prerogative.

Unfortunately, whether IOD is a disease on its own, or a consequence of one or a variety of other diseases, remains unknown to date. This leaves the theoretical possibility that reducing dietary iron

levels, or body iron stores by phlebotomy, has little benefit for the animal unless the underlyingcause is remedied, but also the theoretical possibility that reducing dietary iron levels, and body iron stores by phlebotomy, is an important contribution to preventative black rhino health.

Black rhino iron status: IOD has been identified in browser rhinoceros species (e.g. black rhino, *Diceros bicornis*; Sumatran rhino, *Dicerorhinus sumatrensis*), but not at relevant frequencies in grazing rhino species (e.g. White rhino, *Ceratothenum simun*; Greater one horned rhino, *Rhinocerus unicornis*). The hallmark of IOD of captive black rhinos are, in live animals, dramatically higher serum measures of iron status, in particular documented for ferritin but also serum iron, and – unfortunately – to a much lesser extent for transferrin saturation - as compared to free-ranging specimens (Paglia, 2012; Miller 2016; Wojtusik 2018). At necropsy, the hallmark is the high incidence of iron inclusion in various organs in the form of hemosiderosis or hemochromatosis (Paglia 2012; Olias 2012) as also evident in the necropsy data displayed above, or in the form of high iron levels in the liver (Dierenfeld 2005).

Diagnostics: IOD can be diagnosed in humans by assessing the serum ferritin levels or transferrin saturation. As ferritin is a species specific protein, there is no validated assay in Europe for rhino currently. In the US a validated ferritin test is available. However, irrespective of the enormous difference in ferritin between free-ranging and captive black rhinos, ferritin did not track IOD-related conditions in captive black rhino and is currently not recommended for monitoring iron status or diagnosing IOD (Wojtusik, 2018). Unfortunately, no similar data is available on transferrin saturation, which would be very important. Iron overload in rhinos in Europe is currently diagnosed and monitored best by measuring the iron serum concentration and total iron binding capacity and calculating transferrin saturation (TS). Although concise data are lacking, TS values above 80% should best be considered dangerous.

At necrospy, special stains for iron status (e.g., Prussian blue) should be clearly requested, and liver tissue should be (stored frozen and) analyzed for iron content.

Potential consequences: As known from humans, long term iron overload increases the risk of developing various kinds of organ failure (liver and bone marrow are the most targeted organs) and fertility problems (damaged testis tissue in male rhinos leading to infertility, and irregular oestrous cycle in female rhinos) (Olias, 2012).

Potential causes: Iron homeostasis is a multifactorial process, and the cause of IOD in black rhinos is still not clear. One of the findings (Olias, 2012) was a S88T polymorphism in the HFE gene, suggesting a potential genetic basis of increased iron absorption from intestinal contents in the black rhino as compared to many other mammals. It was suggested that due to the presence of iron chelators in the natural diet (mainly tannins), black rhinos might not have had to evolve mechanisms to protect themselves against excessive iron absorption (Helary, 2012).

Further details including diagnostics and treatment for IOD can be found in Appendix 5.

2.8.3 Preventative medicine

Vaccination

In some areas where the disease is endemic or increased risk factors are identified for the disease then the following annual vaccinations are recommended for black rhino:

- Leptospirosis using leptospiral bacterin with appropriate serovars. Cases of post vaccine reactions have been noted (two cases of weakness and one case of skin sloughing) (Miller, 1996)
- Clostridia, using a multivalent inactivated vaccine
- Cowpox vaccination has been recommended for rhino in collections experiencing cowpox cases.

Training

Animals should be trained to permit sampling and examination. Training can also be beneficial to reduce stress- e.g. during medical procedures. Please refer to previous section in this text, where more details are provided.

Weight

Weighing scales should be incorporated into the enclosure of black rhino so their weights can be regularly recorded and monitored.

Routine health monitoring

This is recommended where it can be carried out without anaesthesia. The following should be part of health exams either carried out conscious or during sedations for health reasons:
Full physical exam not forgetting an oral exam to check for dental problems/ gingivitis.
Blood sample for haematology, biochemistry, serum protein electrophoresis, vitamin E level, fibrinogen, and parameters to test iron status.
Feet should be radiographed if any abnormalities are detected (e.g. nail cracks, pododermatitis).
Rhino should have a microchip placed at the left ear base if there is not one already present.

Parasite monitoring

Faecal samples should be collected twice a year for direct, flotation and sedimentation examinations to look for internal parasites.

Hormonal monitoring

Hormonal analysis from faecal samples should be used to detect oestrus cycles and pregnancy (Radcliffe, 1997). This can be carried out at several laboratories in Europe including Chester Zoo in the UK and Rotterdam Zoo in the Netherlands and the University of Zurich. Hormonal analysis is an important and urgent first step for any animals that are not breeding successfully.

Post mortem examination

Any rhino that dies or is euthanased should have a complete post mortem examination carried out and tissues should be submitted for histopathology to a pathologist with experience in zoological medicine. The pathologists will be able to look for typical problems in black rhino and carry out Prussian blue staining of the liver to assess iron deposition.

2.8.4 Restraint

Physical restraint

Many facilities have a variety of stall and chute designs for physical restraint of black rhino. These should be incorporated into any newly designed facilties.

Chemical restraint

Rhinoceros anaesthesia should only be carried out with experienced personnel (both veterinary and keepers) present. Attention must be given to logistics, planning and preparation for the procedure including contingency plans for emergency.

For animals that are going to be given drugs by remote injection long needles should be used - 60mm long and 2mm wide. The needles used should be straight and without barbs or collars. Nylon darts should be used in preference to metal as they are less traumatic. The most commonly used site for darting is the lateral neck. Darts must be perpendicular to the skin as the thick skin of the rhino makes an angled shot ineffective.

Sites for hand injection in black rhino include the caudal thighs and caudal to the ear. There are safety concerns about hand injecting the potent opioids often used for black rhino anaesthesia.

Equipment needed for chemical restraint includes ropes, a blindfold for the rhino (to prevent corneal abrasion, dirt entering the eye and retinal damage due to bright sunlight), ear plugs (usually cotton wall placed into the rhino's ears when recumbent), mats/ padding for the floor (if the substrate is hard) and adequate equipment for moving the rhino in an emergency situation, e.g. if the animal lies down in a corner in such a way that the airways cannot be monitored. Large volumes of water should

be available and suitable equipment to fan the rhino with in case it becomes hyperthermic. If potent opiates are used personal protective equipment, opiate antagonists and other protective measures should be available for the personnel involved,

Preparation for anaesthesia

Rhino should always be weighed before an anaesthetic to avoid the complication of under or over dosing (Adams, 2005).
The rhino should be starved for 24 hours and water should be withheld for 2 hours before the procedure.

Positioning during anaesthesia

Lateral recumbency provides optimal circulation to the limbs of the rhino, although sternal recumbency may provide better ventilation (Morkel, 2010). Limbs should be 'pumped' every 20 minutes during anaesthesia to encourage circulation (Radcliffe, 2007). Any black rhino that has undergone any exertion before anaesthesia should be placed in lateral recumbency and have their legs pumped for at least a few minutes.

Anaesthetic monitoring

Temperature: For every 1°C rise in body temperature there is a 10% rise in oxygen consumption (Radcliffe, 2014). If the rhino's body temperature rises above 38.5°C the rhino should be liberally doused with water and cooled by fanning. If the rhino's temperature rises above 39°C the procedure should be quickly finished and the rhino woken up. If the rhino's temperature rises above 41°C the rhino should be given drugs to reverse its sedation immediately. The inner ear seems to be an important site for cooling and so in rhino predisposed to hyperthermia the ear plugs should be removed.

Pulse oximetry readings can be difficult to obtain due to the thickness of a black rhino's skin. Probes can be placed on ear pinnae (these can be scraped with a sharp blade to remove the epidermis), mucosal folds of the penis, vulva or rectum.

Capnography should ideally be used for early detection of hypoxia or circulatory failure (Morkel, 2010).

Blood pressure can be measured indirectly using a human blood pressure cuff around the base of the tail.

There is limited information about the use of ECGs in rhino and they are not routinely used during anaesthetic monitoring (Jayasinge, 1992).

Heart rate can be monitored either with a stethoscope or by monitoring a pulse - either the caudal artery of the tail or the medial auricular artery inside the ear. Expected heart rate under anaesthesia is 55-80 beats per minute although in young animals heart rate is much faster and may be up to 140 beats per minute. Capillary refill time is most easily measured on the gum and should be less than two seconds.

Respiratory rate is extremely important to monitor in anaesthetised rhino as respiratory depression is the most significant life-threatening complication seen during routine anaesthesia (Bush, 2005). This is partly due to the rhino's large size and abdominal organs pressing on the diaphragm.
At the start of anaesthesia both nostrils should be checked to ensure they are clear of obstructions. Respiratory rate should be 10-15 breaths per minute at induction, reducing to 4-8 breaths per minute about 10 minutes post induction if potent opioids are used (Radcliffe, 2014). If respiratory rate decreases to four breaths per minute doxapram hydrochloride 200mg can be given intravenously. If respiratory rate decreases further or the rhino becomes hypoxic (SPO_2 less than 80%) then the opiates should either be partially reversed with 5mg nalorphine or butorphanol, or completely reversed with naltrexone.

Standing sedation

The safety of both staff and the animal should be considered when planning a standing sedation. If the sedation is inadequate, stress can cause the rhino to become hyperthermic, so body temperature should be closely monitored. Suggested dose rates for adults are below:

Chemical restraint drug(s)	Reversal agent(s)	Reference
0.5-0.85mg etorphine IM per adult rhino	Naltrexone 50mg/mg etorphine IM	Miller, 2015
2-2.5mg etorphine +10mg detomidine + 15mg butorphanol IM per adult rhino	Naltrexone 40g/mg etorphine IM Atipamezole 5mg/mg detomidine IM	Portas, 2004
25-50mg butorphanol IV per adult rhino	Naltrexone 2.5mg/mg butorphanol IM	Radcliffe, 2000 (in leukaemia paper)
20-30mg butorphanol + 20-50mg detomidine IM per adult rhino	Naltrexone 2.5mg/mg butorphanol IM Atipamezole 5mg/mg detomidine IM	Miller, 2015

General anaesthesia

Potent opioids are most commonly used to anaesthetise rhino. They are often combined with other drugs to provide muscle relaxation and to counteract the hypertensive effect of opioids (LeBlanc, 1987). Opioids alone can provide inadequate relaxation for procedures such as dentals when the mouth needs to be opened.

When anaesthetising a rhino rapid induction is desirable to prevent hyperthermia. Black rhino are predisposed to excitation during induction with etorphine (Portas, 2004), especially when usingremote drug delivery. Training rhino for hand injection can remove this excitatory phase and decrease the total amount of opioid given. However, there are safety concerns about hand injecting opioids. In rhinos that are not trained it may be desirable to give the rhino an oral pre-medication with acepromazine at a dose of 0.07-0.14mg/kg PO 1 hour before the procedure is due to start. The dose rate should be varied within the given range depending on the rhino's stress level and temperament. During the induction phase with opioids black rhino seem to try to stabilise themselves by wedging their head/ horn into any appropriately sized gap or space between bars. This can be extremely dangerous if the rhino tries to become recumbent while its head is wedged. Black rhino should be anaesthetised in stalls without such gaps, or staff should be available to push the rhino's head back out of a gap if necessary.

Oxygen supplementation should always be carried out with rhino under general anaesthesia. It can be given by intratracheal intubation or nasal insufflation at flow rates of 15-30/litres/ min. Oxygen is commonly administered via a flexible nasogastric tube (9-14mm diameter inside and 2m long) attached to an oxygen cylinder with a regulator and flow rate.The tube is passed through a nasal meatus. This has been shown to increase oxygen saturation (Morkel, 2010) and increase anaesthetic safety (Bush, 2004; Fahlman, 2004).

Commonly used drug protocols for adult black rhino are listed below:

Chemical restraint drug(s)	Reversal agent(s)	Reference
1.5-2mg etorphine +2-3mg medetomidine IM per adult rhino	Naltrexone 30mg/mg etorphine IM Atipamezole 5mg/mg medetomidine IM	Miller, 2015
2.5-3mg etorphine + 60mg azaperone IM per adult rhino	Naltrexone 40g/mg etorphine IM	Portas, 2004
1.5-2mg etorphine IM per adult rhino	Naltrexone 30mg/mg etorphine IM	
120-150mg butorphanol + 5-7mg medetomidine IM per adult rhino	Naltrexone 2.5mg/mg butorphanol IM Atipamezole 5mg/mg detomidine IM	Miller, 2015

Black rhino calves can be sedated with 0.05mg/kg detomidine and 0.15mg/kg butorphanol IM. This can be reversed with naltrexone 2.5mg/mg butorphanol IM and atipamezole 5mg/mg detomidine (Miller, pers comm. 2017)

Tranquilisers/ sedatives

Black rhino are very prone to stress and they should be given tranquilisers during transport to stop excessive struggling and associated trauma. Suggested doses are below:

- Azaperone 20-60mg per adult rhino (Kock, 2006)
- Azaperone 40mg per adult rhino (Morkel, pers comm.)
- Detomidine 2-4mg per adult rhino (Kock, 2006)
- Diazepam 70mg PO bid per adult rhino (Morkel, pers comm.)

Crating

Ideally, black rhino should be trained for transfer into a crate. However, if this is not possible they can be given a low dose of opioids and then flagged or called into the crate. Highly-strung or aggressive rhino can be given oral pre-medication with acepromazine 1 hour before the procedure (doses as for general anaesthesia above). The rhino should be given the opioid (usually by remote injection) and then left quietly in its stall for about 7 minutes. After this time, the door to the crate should be opened and the rhino called. The rhino is likely to follow a voice that it is used to into the crate. The rhino is also likely to follow a white flag or feed bag into the crate. Once the rhino is in the crate the opioid is not normally fully reversed to give the rhino a low level of sedation for the transport. If the level of sedation is too great naltrexone can be given into the base of the ear:

- Adult female black rhino etorphine dose for crating: 0.3-0.5mg IM
- Adult male black rhino etorphine dose for crating 0.4-0.6mg IM

Dose rates should be varied according to both the size of the rhino and also its temperament and stress level.

2.8.5 Euthanasia

Euthanasia is best performed on a rhino that has been heavily sedated. Quinalbarbitone should then be injected into one of the larger veins (e.g. medial radial vein) at a dose rate of 40mg/kg IV.

2.8.6 Formulary

Apart from some anaesthetic drugs and some vaccines, no pharmacokinetic studies have been performed in rhinos. Most clinicians base their doses on those for horses but recommended NSAID doses are actually lower. Many black rhino have been found to have gastric ulceration at post mortem examination and it is recommended to use a gastroprotectant such as omeprazole.

ANTIBIOTICS

Drug	Dose	Reference
Trimethoprim sulphas	30-33mg/kg PO sid	Murray, 1999
Enrofloxacin	2. 5mg/kg PO sid	Love, 2017
Cephalexin	25mg/kg PO bid	Murray, 1999
Amoxicillin	28mg/kg PO bid	Murray, 1999
Ampicillin	8mg/kg IV q 6h	Love, 2017
Amikacin	8mg/kg IM sid or 25mg/kg IV sid	Love, 2017
Florfenicol	29mg/kg IM every 4 days	Love, 2017
Metronidazole	oral 15mg/kg BW BID	

ANTIFUNAL AGENTS

Drug	Dose	Reference
Voriconazole	7mg/kg PO sid	Bishop, 2016

ANTIVIRAL AGENTS

Drug	Dose	Reference
Famcyclovir	750mg PO sid for an adult female	Eulenberger, 2015

ANTIPARASITIC AGENTS

Drug	Dose	Reference
Fenbendazole	2.5-5mg/kg PO sid for 3 days	
Ivermectin	0.2mg/kg PO	

ANALGESIC AGENTS

Drug	Dose	Reference
Suxibuzone	3-4mg/kg PO SID or BID	
Phenylbutazone	2mg/kg PO SID or BID	
Flunixin	1.1mg/kg PO sid	Love, 2017
	0.5-1.g/kg IV sid	Kottwitz, 2016
	0.5-1.1mg/kg IM sid	Kottwitz, 2016
Meloxicam	0.5mg/kg PO SID	
Tramadol	0.8-3mg/kg PO bid	Kottwitz, 2016

GASTRIC PROTECTION

Drug	Dose	Reference
Omeprazole	4mg/kg PO sid or 0.25mg/kg IV q 6h	Love, 2017

ANTIHISTAMINE

Drug	Dose	Reference
Hydroxyzine	0.5mg/kg PO bid for 3 days, then 0.75mg/kg PO bid for 3 days and then 1mg/kg PO bid for the remainder of the course	Bishop, 2016

CORTICOSTEROIDS

Drug	Dose	Reference
Dexamethasone	0.1mg/kg PO sid for 3 days, then 0.08mg/kg PO sid for 3 days, then 0.06mg/kg PO sid for 3 days, then 0.04mg/kg PO sid for 3 days, then 0.02mg/kg PO sid for 3 days	Bishop, 2016

OTHER

Drug	Dose	Reference
Phosphorus	10-24g elemental phosphorus PO sid until normal serum levels are re-established	Gillespie, 1990

3 Glossary

AZA	American Zoo Association
CITES	Convention on International Trade in Endangered Species
EAZA	European Association of Zoos and Aquaria
EEP	European Endangered Species Program
IUCN	International Union for Conservation of Nature
JAZA	Japanese Association of Zoos and Aquaria
IATA	International Air Transport Association
SADC	South African Development Community
SSCJ	Species Survival Committee in Japan
SSP	Species Survival Plan
TAG	Taxon Advisory Group

4 References

4.1 Books

Baarde, D.B. and Goede, M.P.M. de 2001, Basisboek methode en technieken, Wolters-Noordhoff, Groningen/Houten.

Emslie, R. and Brooks, M. 1999, African Rhino: Status Survey and Conservation Action Plan, IUCN/SSC African Rhino Specialist Group, IUCN Gland, Switzerland and Cambridge.

Fowler, M.E. and Millar R.E. 2003, Zoo and Wild Animal Medicine, 5th edition, Elsevier science (USA), St. Louis, Missouri.

Gage, L. J. 2002, Hand-rearing wild and domestic animals, 1st edition, Iowa State University Press, Iowa.

Göltenboth, R. and Klös, H.G. 1995, Krankheiten der Zoo und Wildtiere, Black well Wissenschafts-Verlag, Berlin.

Hutchins, M and Kleiman, D.G. and Valerius G. and McDade, M.C. 2003, Grzimek's Animal Life Encyclopedia, 2nd edition, Gale Group, Farmington Hills.

Macdonald, D. 2004, The new encyclopedia of mammals, 2nd edition, Oxford University Press, Oxford.

Nowak, R.M. 1999, Walker's mammals of the world, 6th edition, The Jones Hopkins University Press United States of America, Baltimore.

Stevens, C. E. and Hume, I. D. 1995, Comparative Physiology of the Vertebrate Digestive System, 2nd ed, New York: Cambridge University Press.

4.2 Publications

Abou-Madi N, Coville BR, Olsen JH, Miller AM. 1996. Management of a rectal prolapse in a juvenile black rhinoceros (*Diceros bicornis)* Proceedings AAZV 1996. p421-424.

Adams, W. et al. 2005. Overdose during chemical restraint in a Black rhinoceros (*Diceros bicornis*); Veterinary Anaesthesia and Analgesia.

Amin, R. and Thomas, K. and Emslie, R.H. and Foose, T.J. and Van Strien, N. 2006 'An overview of the conservation status of and threats to rhinoceros species in the wild', International Zoo Yearbook. 40, pp 96-117.

Beagley JC, Lowder MC, Langan JN, et al. 2010. Dental conditions of captive black rhinoceros (*Diceros bicornis*), Proc AAZV AAWV Joint Confer 2010. p138.

Ben-Ari, E.T. 2001. Zoo biologists are taking a scientific approach to improving the quality of life for captive animals; BioScience March 2001 / Vol. 51 No. 3.

Berkeley, E.V., Kirkpatrick, J.F., Schaffer, N.E., Bryant, W.M., Threlfall, W.R., 1997. Serum and fecal steroid analysis of ovulation, pregnancy, and parturition in the black rhinoceros (*Diceros bicornis*). Zoo Biology 16, 121-132.

Bertschinger H.J. 1994. Proceedings of a Symposium on "Rhinos as Game Ranch Animals". Onderstepoort. Reproduction in Black and White Rhinos: A Review.

Beutler E, West C, Speir, JA, Wilson IA, Worley M.. The hHFE gene of browsing and grazing rhinoceroses: possible site of adaptation to a low-iron diet. Blood Cells Mol Dis. 2001;27:342–350.

Bhagwandin A, Haagensen M, Manger PR. 2017. The brain of the black (*Diceros bicornis*) and white (*Ceratotherium simum*) African rhinoceros: Morphology and volumetrics from magnetis resonance imaging. Frontiers in Neuroanatomy. 11(74):1-12.

Bishop GT, Zuba JR, Pessier AP, Hopper J, Kendall G, Rosychuk RAW, Magdesian G. 2016. Medical management of recurrent eosinophilic granuloma in two black rhinoceros (*Diceros bicornis*). J. Zoo Wildl. Med. 47(3):855-861.

Brian, C., Forge, O. and Erb, P. 1999. 'Lion predation on Black rhinoceros (*Diceros bicornis*) in Etosha National Park' *African Journal of Ecology* 37, pp 107-109.

Brown, J.L., Bellem, A.C., Fouraker, M., Wildt, D.E., Roth, T.L., 2001. Comparative analysis of gonadal and adrenal activity in the black and white rhinoceros in north America by noninvasive endocrine monitoring. Zoo Biology 20, 463-486.

Bruins-van Sonsbeek GR. 2018. Monitoring and Treatment Program: Iron storage disease in black rhino (*Diceros bicornis*). 2018 Joint EAZWV/AAZV/Leibniz-IZW Conference Proceedings, Prague. p180.

Bryant B, Blyde D, Eamens G, et al. 2012. *Mycobacterium avium* subspecies *paratuberculosis* cultured from the feces of a Southern black rhinoceros (*Diceros bicornis minor*) with diarrhea and weight loss, J Zoo Wildl Med 43:391.

Bush M, Citino SB, Grobler D. 2005. Imrpoving cardio-pulomnary function for a safer anesthesia of white rhinocceros (*Ceratotherium simum*): use of opiate cocktails to influence receptor effects. 2005 Prooceedings AAZV/AAWV/AZA/NAG joint conference. p259-260.

Bush M, Raath JP, Grobler D, Klein L. 2004. Severe hypoxaemia in field-anaesthetised white rhinoceros (*Ceratotherium simum*) and effects of using tracheal insufflation of oxygen. Jl S.Afr.vet.Ass. (2004) 75(2):79–84.

Carlstead et al (1999); 'Black rhinoceros (*Diceros bicornis*) in U.S. Zoos: II. Behaviour, breeding success and mortality in relation to housing facilities'; Zoo Biology 18, pp 35-52.

Christensen, B.W., Troedsson, M.H.T., Young, L.J., Olivia, M., Penfold, L.M., 2009. Effects of sociosexual environment on serum testosterone in captive male African rhinoceros. Theriogenology 71, 1105-1111.

Claus, M and Hatt, J. M. 2006. 'The feeding of rhinoceros in captivity', International Zoo Yearbook 40, pp 197-209.

Clauss, M., E. Dierenfeld, J. Giff, K. Klasing, L. Kouton, S. Lavin, S. Livingston, B. Nielson, M. Schlegel, K. Sullivan, E. Valdes, A. Ward. 2012. IOD in Rhinos – Nutrition group report: Report from the nutrition working group of the international workshop on iron overload disorder in browsing rhinoceros (February 2011). J. Zoo Wildl. Med. 43(3): s108-113.

Dennis PM, Funk JA, P. Rajaja-Schultz ES, Blumer ES, Miller E, Wittum TE, Saville WJA. 2007. A review of some health issues of captive black rhinoceroses (*Diceros bicornis*). J. Zoo Wildl. Med. 38(4):509-517.

Dierenfeld ES, Atkinson S, Craig M, Walker KC, Streich WJ, Clauss M. 2005. Mineral concentrations in serum/plasma and liver tissue of captive and free-ranging rhinoceros species. Zoo Biology 24:51-72. Dierenfeld, E.S. (1996); Nutrition. In: *Rhinoceros SSP Husbandry Manual*. Fort Worth Zoological Park, Fort Worth, Texas.

Dittrich, L., 1967. Breeding the black rhinoceros *Diceros bicornis* at Hanover Zoo. International Zoo Yearbook 7, 161-162.

Dorsey CL, Dennis P, Fascetti AJ, Wood T, Brown J. 2010. Hypoaminoacidemia is not associated with ulcerative lesions in black rhinoceros, *Diceros bicornis*. J. Zoo Wildl. Med. 41(1):22-27.

Duck, KA, Connor JR. 2016. Iron uptake and transport across physiological barriers. Biometals, 29:573–591.

Duncan AE, Lyashchenko K, Greenwald R, Miller M, Ball R. 2009. Application of ElephantTB STAT-PAK assay and MAPIA (multi-antigen print immunoassay) for detection of tuberculosis and monitoring of treatment in black rhinoceros (*Diceros bicornis*). J. Zoo Wildl. Med. 40:781-785.

EAZA yearbook (1995); Husbandry guidelines for rhinoceros; EEP yearbook Vol.1994-1995 pp. 364-377.

Edwards, K. L., 2013. Investigating population performance and factors that influence reproductive success in the eastern black rhinoceros (*Diceros bicornis michaeli*). Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy.

Emslie, R. 2006. 'Rhino notes: Rhino population sizes and trends' Pachyderm 41, pp 100-105.
Espie IW, Hlokwe TM, Gey van Pittius NC, et al. 2009. Pulmonary infection due to *Mycobacterium bovis* in a black rhinoceros (*Diceros bicornis minor*) in South Africa, J Wildl Dis 45:1187.

Eulenberger K, Bernhard A, Nieper H, Hoffmann K, Scheller R, Meyer H, Zimmerman P, Essbauer S, Pfeffer M, Kiessling J. 2005. An outbrak of cowpox virus infection in a black rhino (*Diceros bicornis*) at Leipzig Zoo. Verh ber Erkrg Zootiere 42:77-85.

Fahlmann A, Foggin C, Nyman G. 2004. Pulmonary gas exchange and acid-based status in immobilized black rhinoceros (*Diceros bicornis*) and white rhinoceros (*Ceratotherium simum*) in Zimbabwe. Proceedings AAZV, AAWV, WDA joint conference 2004. P519-521

Felts, A. 2007. Facility focus – Colombus Zoo; Rhino keeper association volume 1 Issue 4, pp 2-6.
Fleming, GJ, Citino SB. 2003. Suspected vitamin D_3 toxicity in a group of black rhinoceros (*Diceros bicornis*), Proc AAZV, NAG Joint Confer 2003, p34.

Foose, T.J. and Wiese, R.J. 2006. Population management of rhinoceros in captivity; International Zoo Yearbook Vol. 40; pp. 174-196.

Fouraker M, Wagener T. 1989. AZA Rhinoceros Husbandry Resource Manual.

Fouraker, M. and Wagener, T. 1996. AZA Rhinoceros Husbandry Resource Manual; Fort worth Zoological park, Fort Worth, Texas.

Garnier, J.N., Holt, W.V., Watson, P.F., 2002. Non-invasive assessment of oestrous cycles and evaluation of reproductive seasonality in the female wild black rhinoceros (*Diceros bicornis minor*). Reproduction 123, 877-889.

Gaskin JM, Jorge MA, Simpson CF, Lewis AL, Olson JH, Schobert EE, Wollenman EP, Marlowe C, Curtis MM. 1980. The tragedy of encephalomyocarditis virus infection in zoological parks of Florida. Proceedings American Association of Zoo Veterinarians 1980. p1–7.

Gatz, V. (1998); Training für Zootiere: ein Leitfaden zum Training mit dem Operant Conditioning System; Schüling Verlag, Münster.

Goddard, J., 1967. Home range, behaviour, and recruitment rates of two black rhinoceros populations. East African Wildlife Journal 5, 133-150.

Godfrey RW, Dresser BL, Campbell BJ. 1990. Tuberculosis testing of captive rhinoceros. Proc Am Assoc Zoo Vet Ann Meet 1990. p353-354.

Guldenschuh, G. and von Houwald, F. 2002. Husbandry manual for the greater one-horned or Indian rhinoceros; published by Basel Zoo.

Haffey MB, Pairan RD, Reinhart PR, et al. 2008. Urinalysis in three species of captive rhinoceros (*Rhinoceros unicornis, Dicerorhinus sumatrensis*, and *Diceros bicornis*), J Zoo Wildl Med 39:349.

Harrison TM, Stanley BJ, Sikarskie JG, et al. 2011. Surgical amputation of a digit and vacuum-assisted-closure (V.A.C.) management in a case of osteomyelitis and wound care in an eastern black rhinoceros (*Diceros bicornis michaeli*), J Zoo Wild Med, 42:317.

Helary SF, J.A. Shaw, D. Brown, M. Clauss, N.Owen-Smith. 2012. Black rhinoceros (*Diceros bicornis*) natural diets: comparing iron levels across seasons and geographical locations. J. Zoo Wildl. Med. 43(3):848-854.

Hermes R, Hildebrandt TB. 2012. Rhinoceros theriogenology. In Miller RE, Fowler ME, editors: Fowler's Zoo and Wild Animal Medicine Current Therapy ed 7, St. Louis, Elsevier. p546-561.

Hermes R, Saragusty J, Moser, I, Barth S, Holtze S, Lécu A, Cracknell J, Williams D, Göritz, F, Hildebrandt TB. 2018. Differential detection of tuberculous and non-tuberculous mycobacteria by qPCR in lavage fluids of tuberculosis-suspicious white rhinoceros. PLOS ONE 2018, in press.

Hilsberg-Merz S: Infrared thermography in zoo and wild animals. In Fowler ME, Miller RE editors: Zoo and Wild Animal Medicine, 6thed., St. Louis, 2008, Saunders. P20-32.

Hindle, J.E., Mostl, E., Hodges, J.K., 1992. Measurement of urinary estrogens and 20-alpha-dihydroprogesterone during ovarian cycles of black (*Diceros bicornis*) and white (*Ceratotherium simum*) rhinoceroses. Journal of Reproduction and Fertility 94, 237-249.

Hitchins, P.M., Anderson, J.L., 1983. Reproduction, population characteristics and management of the black rhinoceros *Diceros bicornis minor* in the Hluhluwe/Corridor/Umfolozi Game Reserve Complex. South African Journal of Wildlife Research 13, 78-85.

Jayasinge JB, Silva V. 1972. Electrocardiographic study on the African black rhinoceros, Brit Vet J 128

Karlstam E, Ågren E, Möller T, Ramis G, Bölske G, Röken B, Lyashchenko K, Gavier-Widén D. 2015. *Mycobacterium tuberculosis* infection in captive white rhinoceroses (*Ceratotherium simum simum*). J Comp Pathol. 152:54.

Kenny DE. 1999. Salmonella spp. Survey captive rhinoceros in US Zoological institutions and private ranches. J. Zoo Wildl. Med. 30(3):383-388.

Kock ND, Kock MD, Young KB. 1994. Hepatopathy in two black rhinoceros (*Diceros bicornis*) in Zimbabwe: Creosote toxicosis?. J. Zoo Wildl. Med. 25(2):270-273.

Kock, N, Foggin C, Kock MD, and Kock R. 1992. Hemosiderosis in the black rhinoceros (*Diceros bicornis*): A comparison of free-ranging and recently captured with translocated and captive animals. J. Zoo Wildl. Med. 23:230-234.

Kottwitz J, Boothe M, Harmon R, Citino SB, Zuba JR, Boothe DM. 2016. Results of the megavertebrate analgesia survey: elephants and rhino. . J. Zoo Wildl. Med. 47(1):301-310.

Le Blanc PH, Eicker SW, Curtis M, Bochler B. 1987. Hypertension following etorphine anesthesia in a rhinoceros (*Diceros simus*). J. Zoo Wildl. Med. 18(4):141-143.

Lewis S, Duncan M, Houck ML, Bloch R, Haefele H. 2016. Congenital cleft palate and cardiac septal defects in a neonatal southern black rhinoceros (*Diceros bicronis minor)*. J. Zoo Wildl. Med. 47(3):876-878.

Love D, Madrigal R, Cerveny S, Raines J, Rideout B, Lung NP. 2017. Case series: CLinical Salmonellosis in four black rhinoceros (*Diceros bicornis*) calves. J. Zoo Wildl. Med. 48(2):466-475.

McElligott et al. 2004. Interaction between ox peckers and Black rhinos in captivity; Zoo Biology Vol. 23 pp. 347-354.

Mehrdadfar F. 2002. Rhino Keepers' Workshop 2001 Husbandry Survey; Proceedings of the second Rhino Keepers' Workshop 2001, pp 17-18.

Miller E. 1996. AZA Rhino guidelines

Miller M, Buss P.E., Chileshe J., Goosen W., Roos E., van Helden P., Parsons SDC.. 2018. Novel assays for detection of *Mycobacterium bovis* infection in free-ranging African rhinoceros (*Diceros bicornis, Ceratotherium simum*) and implications for conservation. 2018 Joint EAZWV/AAZV/Leibniz-IZW Conference Proceedings, Prague. p245.

Miller M, Buss P, van Helden P, Parsons S. 2016. *Mycobacterium bovis* – report of tuberculosis in a free-ranging black rhinoceros (*Diceros bicornis*) in Kruger National Park, South Africa. Emerg Inf Dis. 2 3:557- 558.

Miller M, Chavey PS, Hofmeyr J, Mathebula N, Doering A, Buss P, Olea-Popelka F. 2016. Evaluation of serum ferritin and serum iron in freeranging black rhinoceros (*Diceros bicornis*) as a tool to understand factors affecting iron-overload disorder. J. Zoo Wildl. Med. 47(3):820-826

Miller M, Michel A, van Helden P, Buss P. 2016 Tuberculosis in rhinoceros: an underrecognized threat? Transbound Emerg Dis, 64:1071-1078.

Miller MA, Greenwald R, Lyashchenko KP. 2015. Potential for serodiagnostics of tuberculosisi in black rhinoceros (*Diceros bicornis*). J. Zoo Wildl. Med. 46(1):100-104.

Miller RE: Perissodactylids. In Fowler ME, Miller RE editors: Zoo and Wild Animal Medicine, 3rd ed., St. Louis, 1993, Saunders. p455-476.

Miller RE: Rhinoceridae (Rhinoceroses). In Fowler ME, Miller RE editors: Zoo and Wild Animal Medicine, 5th ed., St. Louis, 2003, Saunders. p558-569.

Miller RE: Rhinoceridae (Rhinoceroses). In Fowler ME, Miller RE editors: Zoo and Wild Animal Medicine, 8th ed., St. Louis, 2015, Saunders. p538-546.

Molenaar FM, Sansbury AW, Waters M, Amin R. 2008. High serum concentrations of iron, transferrin saturation and gamma glutamyl transferase in captive black rhinoceroses (*Diceros bicornis*). Vet. Rec. 162:716-721.

Morkel PvdB, Radcliffe RW, Jago M, duPreez P, et al. 2010. Acid-base balance and ventilation during sternal and lateral recumbency in field immobilized black rhinoceros (*Diceros bicornis*) receiving oxygen insufflation: a preliminary report, J Wildl Dis, 46:236.

Muneuchi I, Sochi C, Ushio K, Yoshizumi K, Hashimoto W. 2018. The treatment of Eastern Black Rhinoceros Suspicious for Hyperferraemia. Jour. Jpn. Assoc. Zoo. Aqu. 60(3):74-83.

Munson L, Koehler JW, Wilkinson JE, Miller RE. 1998. Vesicular and Ulcerative Dermatopahty Resebling Superficial Necrolytic Dermatitis in Captive Black Rhinoceroses (*Diceros* bicornis). Vet.Pathol. 35:31-42.

Munson L, Miller RE: Skin diseases of black rhinoceroses. In Fowler ME, Miller RE editors: Zoo and Wild Animal Medicine, 4th ed., Philadelphia, 1999, W.B. Saunders.

Murnane RD, Raverty SA, Briggs M, Phillips LG. 1994. Chronic recurrent anemia, massive pulmonary and systemic mineralization, chronic interstitial nephritis and membranoproliferative glomerulonephritis, and hemosiderosis with myelophthisis in an euthanized black rhinoceros. Proceedings association of reptilian and amphibians veterinarians 1994. p282-286.

Murray S, Lung NP, Alvarado TP, Gamble KC, Miller MA, Paglia DE, Montali RJ. 1999. Idiopathic hemorrhagic vasculopathy syndrome in seven lack rhinoceros. JAVMA 216(2):230-233.

Mylniczenko ND, Sullivan KE, Corcoran ME, et al. 2012. Management strategies of iron accumulation in a captive population of black rhinoceroses (*Diceros bicornis minor*), J Zoo Wildl Med 43:S83.

Mylniczenko ND, Sullivan KE, Corcoran ME, Fleming GJ, Valdes EV. 2012. Management strategies of iron accumulation in a captive population of black rhinoceroses *(Diceros bicornis minor*). J. Zoo Wildl. Med. 43(3): s83-91.

Nance MB. 1998. Clinical management of severe necrotic laminar disease in an eastern black rhinoceros (*Diceros bicornis michaeli*) associated with an undetermined etiology. Proceedings AAZV/AAWV joint conference 1998. p208-212.

Ndeereh D, Okita-Ouma B, Gayner J, Mutinda M, Gakuya F. 2012. Unusual mortalities of the eastern black rhinoceros *(Diceros bicornis micheali*) duet o clostridial enterotoxaemia in Ol Jogi Pyramid Sanctuary, Kenya. Pachyderm 51:45-51.

Neiffer DL, Klein EC, Wallace-Switalski C. 2001. Leptospira infection in two black rhinoceroses (*Diceros bicornis michaeli*), J Zoo Wildl Med 32:476.

Nordstrom, L.A 2004. Effects of Zoological Enclosures on Rhinos and Tapirs.

Olias P, Mundhenk L, Bothe M, Ochs A, Gruber AD, Klopfleisch R. 2012. Iron overload syndrome in the black rhinoceros (*Diceros bicornis*): microscopical lesions and comparison with other rhinoceros species. J Comp Pathol. 147(4):542-549

Olivier A, Lane E, Volkmann D, Hofmeyr M, Stegmann G. 2001. Rectal prolapse associated with a healed pelvic fracture in a pregnanct free- ranging African black rhinoceros (Diceros bicornis). Part 2: surgery and necropsy. J S Afr Vet Assoc. 72(4):242-244

Paglia DE, Tsu IH. 2012. Review of laboratory and necropsy evidence for iron storage disease acquired by browser rhinoceroses. Journal of Zoo and Wildlife Medicine 43:S92-S104

Paglia DE. 2006. Iron storage syndrome in rhinoceros. Potential role for rhino keepers in prevention and therapy. Proceedings of the Fourth Rhino Keepers workshop 2005 at Columbus, Ohio. p1-10

Paglia, D.E., and P. Dennis. 1999. Role of chronic iron overload in multiple disorders of captive black rhinoceroses (*Diceros bicornis*). Proc. Am. Assoc. Zoo Vet. 1999. p163-171.

Parsons SD, Morar-Leather D, Buss P, Hofmeyr J, McFadyen R, Rutten VP, van Helden P, Miller MA, Michel AL. 2017. The kinetics of the humoral and interferon-gamma immune responses to experimental Mycobacterium bovis infection in the white rhinoceros (*Ceratotherium simum*). Front Immunol. 2017;doi: 10.3389/fimmu.2017.01831.

Pearson H, Gibbs C, Wright AI. 1967. Surgical treatment of a case of rectal prolapse in a young African rhinoceros (*Diceros bicornis*). Vet Rec. 80(17):519.

Pessier AP, Munson L, Miller RE. 2004. Oral, nasal, and cutaneous eosinophilic granulomas in the black rhinoceros (*Diceros bicornis*): a lesion distinct from superficial necrolytic dermatitis, J Zoo Wildl Med 35:1.

Portas TJ, Hildebrandt TB, Bryant BR, Görits F, Hermes R. 2010. Seminoma in a southern black rhinoceros (*Diceros bicornis minor*): diagnosis, surgical management and effect on fertility. Australian Veterinary Journal. 88(1-2):57-60.

Portas TJ:. 2004. A review of drugs and techniques used for sedation and anaesthesia in captive rhinoceros species, Austr Vet J 82:542.

Radcliffe RW, Czekala NM, Osofsky SA. 1997. Combined serial ultrasonography and fecal progestin analysis for reproductive evaluation of the female white rhinoceros (*Ceratotherium simum simum*): preliminary results. Zoo Biology 16:445-456.

Radcliffe RW, Morkel PB: Rhinoceroses. In West G, Heard D, Cauklett N editors: Zoo Animal and Wildlife Immobilization and Anesthesia, Ames, 2007, Blackwell Publishing. p543-566.

Radcliffe RW, Morkel PB: Rhinoceroses. In West G, Heard D, Cauklett N editors: Zoo Animal and Wildlife Immobilization and Anesthesia 2nd edition, Ames, 2014, Blackwell Publishing.

Radcliffe RW, Paglia DE, Couto G. 2000. Acute lymphoblastic leukemia in a juvenile southern black rhinoceros (*Diceros bicornis minor*). J. Zoo Wildl. Med. 31(1):71-76.

Radcliffe RW, Schumacher J, Hartsfield SM, Merritt AM, Murray MJ. 1998. Idiopathic distal esophageal dilation in a southern black rhinoceros (*Diceros bicornis minor*). J. Zoo Wildl. Med. 29(4):465-469.

Radcliffe, R.W., Eyres, A.I., Patton, M.L., Czekala, N.M., Emslie, R.H., 2001. Ultrasonographic characterization of ovarian events and fetal gestational parameters in two southern black rhinoceros (*Diceros bicomis minor*) and correlation to fecal progesterone. Theriogenology 55, 1033-1049.

Reuter, H. -O. and Adcock, K. (1998); Standardised body condition scoring system for Black rhinoceros (*Diceros bicornis*); Pachyderm 26, pp 116-121.

Rieches, R. 1999. A keeper’s guide to the introduction and management of the Indian, black and white rhinoceros; Proceedings of the First Rhino Keepers' workshop 1999, pp. 70-93.

Rodriguez JMM, Chantrey J, Unwin S, Verin R. 2017. Cardia Truncus Arteriosis in an Eastern Black rhinoceros (*Diceros bicronis michaeli*). J. Comp. Path. 157:276-279.

Roth, T.L. 2006. A review of the reproductive physiology of rhinoceros species in captivity; International Zoo Yearbook 40, pp. 130-143.

Schlanser JR, Bohart GW, Paperd DW, Wagner C, Marquardt M, Harrison TM. 2016. Technique for venipuncture of the transverse facial vein in the black rhinoceros (*Diceros bicornis*). Zoo Biology. 9999:1-4.

Schwarz C, Grothmann P, Gottschlak J, Eulenberger K, Einspanier A. 2014. Breeding management of black rhinos (Diceros bicornis míchaeli) in Magdeburg Zoo 2014. Tierärztl Prax 42 (G): l50-155.

Smith, JE, Chavey PS , Miller RE. 1995. Iron metabolism in captive black (*Diceros bicornis*) and white (*Ceratotherium simum*) rhinoceros. J. Zoo Wildl. Med. 26(4): 525-531.

Smith, R.L., Read, B., 1992. Management parameters affecting the reproductive potential of captive, female black rhinoceros, *Diceros bicornis*. Zoo Biology 11, 375-383.

Suzuki Y, Nakajima C. .2015. Mycobacterium orygis–associated tuberculosis in free-ranging rhinoceros, Nepal . Emerg Infect Dis. 2016, 22:570.

Takle GL, Suedmeyer WK, Garner MM 2008: Vitiligo in a sub-adult eastern black rhinoceros (*Diceros bicornis michaeli*), Proc AAZV/AAWV/AZA/NAG Joint Confererence 2007. p243.

Thapa J, Paudel S, Sadaula A, Shah Y, Maharjan B, Kaufman GE, McCauley D, Gairhe KP, Tsubota T, Wack AN, Miller CL, Wood CE, Garner MM, Haefele HJ. 2010. Melanocytic neoplasms in a black rhinoceros (*Diceros bicornis*) and an indian rhinoceros (*Rhinoceros unicornis*). J. Zoo Wildl. Med. 41(1):95-103.

Wagner, D.C. and Edwards, M.S. 2002. Hand-rearing Black and White rhinoceroses: A comparison; Proceedings of the second Rhino Keepers Workshop 2001, pp. 18-27.

Weber M, Miller RE. 1996. Fungal pneumonia in black rhinoceros (*Diceros bicornis*). Proc Am Assoc Zoo Vet Annu Meet; 1996. p34–36.

Wenker C, Wyss F, Hoby S, Ghielmetti G, Friedel U, Gurtner C, Posthaus H. 2018 Non-tuberculous mycobacterial lung infection in an African elephant (*Loxodonta africana*) and a greater one-horned rhinoceros (*Rhinoceros unicornis*) caused by *Mycobacterium avium ssp. hominissuis* and *Mycobacterium nebraskense* and the reaction to ante- and postmortem tests. 2018 Joint EAZWV/AAZV/Leibniz-IZW Conference Proceedings, Prague. p288-290

Wojtusik J, Roth TL. 2018. Investigation of factors potentially associated with serum ferritin concentrations in the black rhinoceros (*Diceros bicornis*) using a validated rhinoceros-specific assay. J. Zoo Wildl. Med. 49(2):297–306.

Yamanishi H, Iyama S, Yamaguchi Y, Kanakura Y, Iwatani Y. 2003. Total iron binding capacity calculated from serum transferrin concentrations or serum iron concentration and unsatured iron/binding capacity. Clin. Chem. 49(1):175–8.

4.3 Online material

Adcock K. and Amin R. (2006); Save the rhino international; Available from: http://www.savetherhino.org; (accessed September 22, 2008).

African Rhino Specialist Group 2003, 2007 IUCN Red List of Threatened Species: *Diceros bicornis*, http://www.iucnredlist.org/search/details.php/6557/summ (accessed July 06, 2008).

Anonymous (2008), "Ungulates and Subungulates" PowerPoint presentation, available from: http://cstl-csm.semo.edu/scheibe/Mammalogy/Ungulates-Subungulates.ppt#256,1,Ungulates and Subungulates (accessed September 26, 2008).

Dollinger, P. and Geser, S. 2008, WAZA's virtual Zoo Black rhinoceros, Available from: http://www.waza.org/virtualzoo/factsheet.php?id=118-003-003-001andview=Rhinos, (accessed: June 24, 2008).

EAZA, 2006; EAZA minimum standards for the accommodation and care of Animals in Zoos and Aquaria; available from www.eaza.net (accessed on September 23, 2008).

Huffman, B 2007, Ultimate Ungulate order Perissodactyla, available from: http://www.ultimateungulate.com/Perissodactyla.html (accessed: June 24, 2008).

IATA, 2007; Live Animals Transportation by AIR; Available from http://www.iata.org/whatwedo/cargo/live_animals/index.htm (Accessed on September 26, 2008).
IRF 2008, International Rhino Foundation, available from: http://www.rhinos-irf.org/black/ (accessed: June 24, 2008).

Jansa, S. 1999, "Diceros bicornis" The Animal Diversity Web, available from: http://animaldiversity.org, (accessed July 02, 2008).

Law, J. and Myers, P. 2004, "Crocuta crocuta" The Animal Diversity Web, available from: http://animaldiversity.org, (accessed July 02, 2008).

Lindsay, N (2002); EAZA Rhinoceros TAG regional collection plan; Available at www.eaza.net.
Massicot, P. (2007), "Animal info – Black rhino" Animal info, available from: http://www.animalinfo.org/species/artiperi/dicebico.htm (accessed September 24, 2008).

Myers, P., R. Espinosa, C. S. Parr, T. Jones, G. S. Hammond, and T. A. Dewey. 2006, The Animal Diversity Web, Available from: http://animaldiversity.org, (accessed July 02, 2008).

Appendix I: Criteria for the body condition scores.

	CONDITION Assessment site	Numerical scale Descriptive scale	5 excellent (heavy)	4 good (ideal)	3 fair (average)	2 poor (thin)	1 very poor (emaciated)
A	Neck	General appearance	thick, well muscled, rounded	well muscled, rounded	rounded	flat, narrow neck; nuchal ligament visible	narrow, angular (bony) neck; nuchal ligament prominent
		Prescapular groove		-	slightly visible	obvious	deep groove very obvious
B	Shoulder	General appearance	well-muscled, rounded	rounded	flat	flat, slightly angular (bony)	angular, bony
		Scapula	covered	covered	spine visible	obvious	very obvious
C	Ribs		well covered (skin folds)	covered (skin folds)	visible	obvious	very obvious
D	Spine	General appearance	rounded	slightly angular	back groove back visible	groove deep obvious	back groove very obvious
		Spinous processes	covered	slightly visible	visible	prominent	very prominent
E	Rump	General appearance	well rounded	flattened	slightly concave	concaveobvious depression	
		Bony protuberances	covered	slightly visible	visible	prominent	very prominent
F	Abdomen	General appearance	distended, taught	filled	slightly tucked in	tucked in	tucked in
		Flank-fold	none	sometimes slightly visible	slightly visible	visible	obvious
G	Tail base		rounded (bulging)	rounded	narrow	slightly bony	very thin and bony

Appendix 2: Faecal collection protocol (Chester Zoo)

Faecal Collection Protocol for Routine Hormone Analysis

For samples to be useful for reproductive monitoring, it is extremely important that they are collected regularly.

- For reproductive monitoring in females, every other day is required.
- For reproductive monitoring in males, weekly samples are sufficient.

Collection of Faeces

Identification:

- The most important requirement is that that you know which animal the sample came from.
- If the animals are not housed separately you will need to observe the animal defecating and collect the sample as soon as possible.
- If you think you may have a problem with identification of faeces it is possible to mark the faecal samples by feeding a marker.

Contamination:

- Avoid faeces that are contaminated with urine

- Urine has hormones too, and this interferes with measurements of faecal hormone concentrations.
- Please also ensure that the faeces are not contaminated with another individual's sample (faeces or urine).

Collection:

- Once you have properly identified the sample, try to collect the sample as soon as possible following defecation
- Hormone concentrations in samples left exposed to the environment for extended periods will decrease the accuracy of measures.
- If animals are housed individually, it is not necessary to observe defcation – collecting the freshest sample from the over-night enclosure first thing in the morning is sufficient.
- There is no need to collect the entire faecal pile/bolus. Instead (as 'pockets' of hormone concentrations can be found within the faecal sample) turn zip-lock bag inside out and collect several (3-4) 'sub' samples from different areas of the same faecal sample.
- In total, collect approximately a handful sized amount of faecal material into the zip-lock bag.
- Label the bag using a <u>waterproof permanent marker</u> (i.e. Sharpie® pen) with:

    - Animal's name
    - Species
    - Date (day/month/year)
    - Time (whether am or pm)

Please note - sample labelling will be easier if zip-lock bags with a write-on panel are used – once samples have been frozen, writing directly onto the plastic bag or using an unsuitable pen can wipe off easily, making samples un-useable.

Storage:

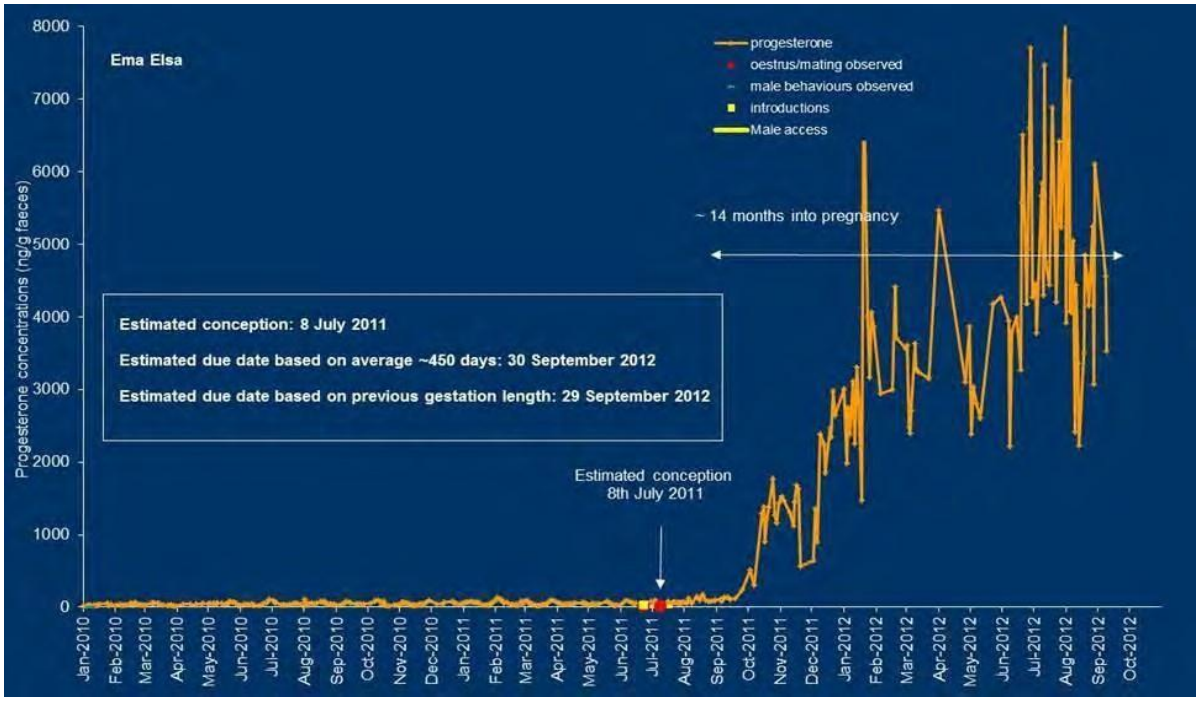
- Store samples as soon as possible after collection in freezer at -20 ^{o}C
- Hormones concentrations will degrade if samples are left out too long – please try to freeze within an hour of collection, or as soon as possible.

Appendix 3: Black rhino birth plan (Chester Zoo 2012)

When is she due?

Timing of parturition, how do we know, what is the window?

Can we predict it more accurately (do we need to?)



Prior to the Birth

1. Enclosure preparation	Female has access to 2 pens both with beds, sand bed in the smaller pen
2. Changes in routine	No change except possibly shut in at night
3. Diet	No change until Calf born then increase Zebra pellet from 2kg daily to 4kg daily and ad lib lucerne

The Birth itself

1. Normal birth data	The following data was obtained from the AZA husbandry manual 1996. • 3 days to a week, female starts bagging up • Within 24 hours the area around eyes goes very dark and sweats • Mother very restless 24 hrs before • Birth usually at night or in early morning • The calf is usually born within 10-12hrs of waters breaking (but can be longer in first time mothers) - generally around 4 hrs • Calf usually born head first • Birth weight 24-41kg

	• House won't be cleaned, food just quickly put in and water checked, house to be closed to public
2. What monitoring of the birth do we plan to do?	• As the female previously had no problems with birthing, it would appear that there is little to be gained from staff being on site during the night. The birth was recorded for two reasons: 1) To gather data on the birthing process 2) to use footage to publicise birth. • Cameras are installed and working in all indoor enclosures. Low level lighting is used to improve image (IR camera also available). Camera outputs are continuously recorded on a hard drive. • Camera footage can also be viewed live on monitor. • During the day if it is not obvious calf has fed, they can be observed from a distance with binoculars.
3. What are the indications, if any, for intervention during the birth	• All staff should stay away from the enclosure during the birth as it is quite likely that any disturbance will delay the process and may well lead to decrease chances of survival of the calf. • Only envisage intervening if the calf was obviously stuck (i.e. part sticking out) and the mother visibly exhausted – in this case we would need to consider anaesthesia to pull the calf out. We discussed use of the chute but if the cow was in distress it is unlikely she would enter it. Vet team to produce contingency plan for this eventuality. OR • If the dam's waters had broken >16hrs with no calf born (we may try and administer oxcytocin - vet team to develop contingency plan).

<u>After the birth – rhino issues</u>

1. Normal calf data	The following data was obtained from the AZA husbandry manual 1996: • Birth weight 24-41kg • Calf normally stands 30min – 5hrs post birth • Calf normally suckles 1-2hrs after birth (longest 16hrs) • Nursing may occur with the dam standing or lying on her side • Calves should be gaining about 4.5kg/day during the first 10days • In the first 2mths, calves usually suckle every hour • After 2mths frequency drops to about every 2.5hrs • Weaning occurs naturally at about 2hrs. Weaning can commence at 6mths however it is recommended that it not be done until 1yr • First defecation occurs 2-10days post birth • Passing of Placenta 1-2 days post birth
2. Have problems been seen at CZ or other institutions?	• Mothers have been seen to push the calf around with horn: this may look aggressive but is normal. • One collection found that several calves walk on their heels for the first few days, no interference was needed.

	• One calf has died from being pushed into a water bowl. • One calf has been hand-reared due to being distressed and weak, mothers milk had dried up. • One female had problems with breech birth, she stopped pushing so was left for 24 hours, unfortunately she must have stopped pushing as the calf had already died.
3. What problems might occur and what actions could be taken.	Possibilities include: • Aggression from mother: action will be to remove the mother and check the calf. • Lack of interest from mother: action would be to remove the mother and check calf. Treat calf (warm up, feed as necessary). If the calf is ok, try to reintroduce and see if dam is now more attentive. • Calf trying to suckle but doesn't seem to be getting sufficient milk: this would become apparent if calf is distressed/restless and/or calf appears weak and/or calf is not gaining weight. Action would be to consider supplementary or hand-feeding (see protocol below).
4. Hand rearing or supplementary feeding protocols	Indications for supplementary feeding: • Weak calf – i.e. if weak from birth and not standing after 4 hours • Not fed in first 12hrs – requirement to get some colostrum into it to ensure not immunosuppressed. Indications for hand rearing: • Dam not sufficient milk – close observation of mammary glands • Dam aggressive or not letting calf suck Hand rearing protocol: Ensure bottles and teats are ready for use. Ensure familiar with sterilisation/cleaning regime? Confirm milk formula: • For the first 24 hours, colostrum is fed – rhino is best, but either cow or horse can be used if required. • For the 2nd 24 hours, the transition from colostrum to formula should be 50:50. • Thereafter, milk formula is used – either rhino milk replacer or cow's milk (+iron/lactose) • Amount of milk given is determined by the calf's body weight. Start feeding a total of 10% of body weight daily, increase to ~15-20% after the first two weeks. • For ease of use, can use Mazuri Rhino milk replacer* which has been formulated to mimic the composition of rhino milk, as described above. It already contains lactose and a vitamin and mineral mix so no further supplementation is required. • Feed every 2 hours to begin, including a late evening feed for first

	week then if strong enough 5pm feed and first thing in morning feed. • Daily weight to be plotted and monitored
5. Weaning and reintegration with other rhinos	• Aim to wean the calf after 6-9 months, depending on progress of the calf.

After the birth – managing information and the public

1. Who do we tell, when and how	• There is no reason to keep the birth a secret however in the first days information should be managed carefully so as to reduce possibility of disturbance to the mother and calf. • Staff can be told mother and calf have bonded well and the calf is suckling well (probably after at least 48hrs). • Press statements should be ready to give to the media - if the birth goes well and mother and calf are well bonded a very limited photo shoot or filming may be allowed.

Appendix 4: EAZA Guidelines: General considerations on tuberculosis testing in zoos

- In the context of screening, using a test with a high sensitivity for screening will result in a greater chance of detection but also an increase in false positives.
- In the context of diagnostics used for treatment / culling decisions, using a test with a high specificity is recommended to avoid treating or killing a non-infected endangered animal. This can however lead to a higher chance of false negative results.
- If an animal tests positive by serology or Interferon Gamma Release Assay (IGRA), initially a minimum of 10%+1 of the herd/group (but **preferably the whole herd/group)** should be screened with the same immunological test.
- If an animal comes up positive on **culture**, the **whole herd/group** should be screened with an immunological test (either serological or IGRA, depending on availability and current scientific backup of these respective tests)
- Decision making for when to treat/euthanase:
    - Mandatory euthanasia if the animal is PCR or culture positive. *Mycobacterium bovis* and *Mycobacterium tuberculosis* are notifiable diseases in several countries in Europe.
    - Based on zoo risk assessment policy/ decision of senior zoo management if the animal is only IGRA/serology positive
- Staff screening (by human IGRA : Quantiferon Gold / TBSpot) should be part of any testing surrounding a known culture-positive animal. Testing is also advisable when a rhino shows increasing serology titres.

Rhino specific tuberculosis testing in zoos

Recommended validation and interpretation of test results

INDIRECT / IMMUNOLOGICAL ASSAYS

Skin testing

- **Single test (SITT):**
  - Location: at the base of the ear pinna or caudal fold, where skin is softer.
  - Recommended tuberculin / dosage: 2500 UI / 0.1 ml bovine tuberculin (bovine PPD).
  - Injection: should be done with a maximum of 25G/20mm needle to remain intradermally.
  - Reading: skin thickness measurement before injection and after 72 hours.
- **Comparative test (CITT):**
  - Location: ear base, one side avian (e.g. left) and contralateral (e.g. right) side with bovine tuberculin to avoid vicinity contact inflammation.
  - Tuberculin: bovine PPD, avian PPD.
  - Dosage is 2500 UI / 0.1ml for each.

Interpretation: Considering the large numbers of antigens included in PPD tuberculins, and given the high exposure of rhinoceros to environmental mycobacterial antigens (increasing the risk of cross reactive bovine PPD skin reactions), only the comparative test (CITT) should be used. Skin test results can be very inconsistent, and the predictive values are suspected to be low; thus even CITT results should be interpreted cautiously, and are more often indicative of any mycobacterial exposure (i.e., to non-tuberculous mycobacteria) when positive.

Serology

- ELISA: screening should be done to detect either a broad antigenic panel such as PPD or preferably for antibodies against relevant purified antigens that are present in both *M. tuberculosis* and *M. bovis*, such as ESAT-6 and CFP-10. Indirect or sandwich ELISA techniques are not species specific and can be used, whereas direct ELISAs that are specific for cattle or sheep antibodies should not be used in Rhinoceros.
- DualPath Platform (©Chembio): This is a rapid test that could be performed in the field and provide results within minutes. Presence of antibodies against 3 antigens can be assessed at once (MPB83; ESAT-6/CFP-10). A bias in visual interpretation may exist between operators and in processing of the test, the manufacturer's instructions should be carefully followed (e.g. serum and conjugate respective volumes). Although some reports show significantdetection in infected black and white rhinos (Parsons, 2017), specificity remains unknown and titer levels are inconsistent and can be low. Therefore, a reflectance reader is recommended to evaluate results of this test, making it more reliable.

Gamma Interferon Test:

- Commercial kits for cattle or humans should not be used (resp. BOVIGAM® and QUANTIFERON Gold ® or TB Spot TB ®) as these are **not validated** and **are not compatible**

with rhinoceros species (the embedded ELISA cannot detect Interferon Gamma of rhinoceroses).

- However, Quantiferon test could be modified by replacing Human interferon ELISA by Equine Interferon ELISA, which seems to cross react with white rhinoceros IFN-gamma (Miller, 2016; Parsons, 2017).
    - This adaptation should be done only by laboratories that are familiar with carrying out IGRAs.
    - Raw optic density results should be available to the veterinarian and officials to discuss, even if cut off values are selected by laboratory to assess positivity.
- A second Gamma Interferon Assay, using a monoclonal antibody able to specifically detect Rhinoceros Gamma Interferon, could be also used (Parsons, 2017).
    - Application of this test requires the lab to get supply of monoclonal antibodies for Rhinoceros gamma interferon
- These two experimental Rhinoceros IGRAs differ regarding the reported antigens selected as immuno-stimulant for:
    - the positive control (PHA for the modified Quantiferon, PMA/Cal for the Rhino specific IGRA)
    - the TB antigens selected (ESAT6-CFP10 proteins for the modified Quantiferon, PPDs for the Rhino-specific IGRA)

DIRECT EXAM

A sample obtained using bronchio-alveolar lavage (BAL), sputum, oesophageal lavage (OL), organs or biopsies can be submitted for culture **and** PCR.

Recommended Pre-Shipment testing

Officially not mandatory if the zoo of origin is BALAI approved and in a country/district/area free of TB. Testing may be skipped if there is no history of TB in the collection and in the contact animals so far.

Recommended Regular screening

Routine post mortem examinations (PMEs) on all ungulates with special focus on Mycobacterium tuberculosis complex, including histopathology of the cervical and thoracic lymph nodes.

Recommended testing in case of suspicion (i.e. positive serological test, nearby culture positive case in Rhinoceros/other species)

As the positive predictive values of indirect tests can be low, and considering the overall very low MTBC prevalence so far reported in rhinos, the decision to cull should never be made based on the results of a single positive immunological (either CMI or humoral assessment) test.

Direct exam of the whole rhinoceros group should be undertaken as soon as possible to establish whether the animal is excreting the organism. This sampling method requires immobilisation and

adapted equipment (endoscope to go through the nostril to the larynx, or intubation to pass scope through oral cavity, long catheters etc..) with experienced personnel.

- Bronchoalveolar lavage (BAL): 150 to 180 ml of sterile saline can be instilled in both main bronchi. BAL could be then processed to centrifugation and re-suspension of obtained concentrate pellet into 4ml.
- Oesophageal lavage: around 50 to 150ml sterile saline. Senstivity is likely lower than the BAL as more remote from infectious burden.
- False-negative culture results can occur in MTBC infected rhinoceros if the lesion doesn't communicate with airways, or the sampling technique does not test all areas of the lung.

Recovered fluids should be screened through PCR and culture for MTB complex organisms. Direct exam with stain is poorly sensitive / specific and should not be considered as diagnostic. However, sensitivity could be increased by preparing a smear out of a pellet of centrifugated BAL and using auramine stain instead of Ziehl-Nielsen stains.

Nasal and oral swabs on conscious and /or trained animals can be taken for culture and PCR while setting up the procedure of immobilization for BAL.

Negative outcomes on PCR/culture = there is no excretion of MTBC on the day of exam; this does not rule out infection. However, serial testing, i.e. several tests following each other all with the same result, will increase specificity.

According to the institution's and official veterinarians' risk assessment, this exam should be renewed once or twice over the first year in case of suspicion, with an interval depending on the risk assessment. Then, if two direct or relevant serological tests turns out negative 6 months apart, surveillance should continue, but the risk of disease and transmission will be considered lower and the frequency of examinations can be reduced. The plan and frequency of testing may be reviewed in case of any availability of a more sensitive / specific test such as gamma interferon test. Moreover, training of the rhinoceros for blood draws should be promoted (chute, staff training), so that monthly or quarterly tests may be considered.

All personnel involved in the examination, immobilization, sampling and PME should be aware that zoonotic transmission is a real risk and should wear proper protective equipment in all hazardous phases of work. Annual TB screening of involved staff is advised.

Treatment of tuberculosis in rhino / euthanasia

The decision of treating or culling a confirmed MTBC infected rhinoceros should be done in discussion with national and local veterinary authorities. The EEP Coordinator/Rhinoceros TAG chair should be informed by the holding institution. See EAZA Culling www.eaza.net/assets/uploads/Position-Statements/ for further guidance.

There are no previous citations of **successful** treatment of rhinoceroses among the five notified reports: therefore no dosages or pharmacokinetic studies of any antimicrobial drugs have been

correlated with a curative outcome. Reports in black rhinoceros (Duncan, 2009) describe either death with positive culture during treatment course or re-occurrence of disease some months after the end of treatment. Hence, vet advisors are generally not recommending treatment, as efficacy and side effects are yet unsure, the risk of relapse is unknown, and the risk of persistent shedding remains placing staff and animals at risk. Attempting treatment should therefore be very carefully considered with respect to costs, compliance, animal welfare and the guarded prognosis.

If the decision has been made to cull, necropsy should be planned beforehand with the help of a pathologist that has experience with tuberculosis cases. Post mortem examination should be as exhaustive as possible on highly relevant tissues (lungs, thoracic and mesenteric lymph nodes, spleen), as micro-granulomas ("pinpoint" lesions, common in white rhino) could be missed both macroscopically and histologically, leading to the wrong affirmation of a negative animal (Karlstam, 2015).

Appendix 5: Management of iron storage disease

At the Rotterdam Zoo, a monitoring and treatment protocol was designed (Bruins-van Sonsbeek, 2018).

Management protocol (Rotterdam Zoo)

Monitoring the animal (in animals <8y at least yearly, >8y 2-4times a year (when transferrin saturation is clearly increasing monthly):

- Haematology: haematocrit (Ht) and hemoglobulin (Hb) in EDTA whole blood (anaemia)
- Chemistry: urea, creatinine, GGT in heparin plasma or serum (renal and liver function)
- Iron values: iron and total iron binding capacity (TIBC) to calculate transferrin saturation
    - In serum:
        - Iron – amount of iron in the blood
        - Total iron binding capacity – (capacity of the blood to bind iron with transferrin)
        - Transferrin saturation – % of protein (transferrin) which is bound to iron, how much buffer capacity is left in the blood? – calculated from serum iron and total iron binding capacity

Monitoring the iron intake/amount of iron in the diet:

- Test every batch of roughage: Analyzing all roughage for iron (separate low iron batches), give branches every day <6000 mg daily uptake [Clauss], our experience <4000 mg and maybe even below 3000mg
- Use pellets with low amount of iron
- Also check your water source if you met all the conditions above and the saturation is increasing

So, if iron saturation >70%, start training for phlebotomy!

NB: If there is no time for training (for instance rhino is very slow, saturation is over 90% and GGT levels are increased, phlebotomy can be done under general anaesthesia. It is very important to check

the oxygen saturation continuously and to give extra oxygen by a tube in the nose during theprocedure. If the oxygen saturation drops, immediately stop the procedure and antagonise the anaesthesia. If the animal is under general anaesthesia, you can use every available vein (so not only front legs but also hindlegs), don't bother however trying to go for the jugular, this is more than 8cm deep). In this way it is possible to withdraw 6.5-8L of blood very rapidly)

Evidently, doing a phlebotomy under general anaesthesia represents a major decision that may meet with serious reluctance. Initiating a training problem when a health hazard is already suspected also represents a stressful situation, with pressure on the trainers, which may impede the outcome. Therefore, we stress once more that routine training should be part of black rhino husbandry routines when animals are considered healthy.

Performing Large Volume Phlebotomy (LVP)

No more than 8-10L in total over 1 month should be withdrawn to avoid the risk of anaemia.

Requirements Large Volume Phlebotomy in Black Rhino

- Training of animals
    - for blood collection
    - for being comfortable around people
    - to stand still for long periods of time
    - keep it positive
- 3 people needed for procedure
    - handling animal, needle control, flow control
    - good communication during procedure essential (flow codes)
- Chute/crush to work safely on the animal
- Heat to dilate the veins
    - warm environment (ca 23°C/73°F)
    - warm water (30-35°C /86-95°F)

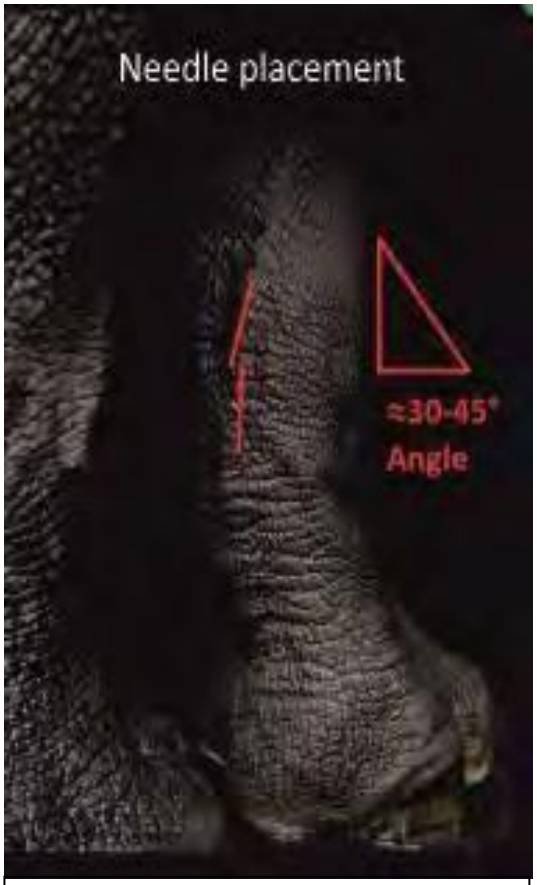


Figure 1. Medial radial vein front leg, best option for standing unsedated phlebotomy

Figure 2. Materials needed for LVP

Materials needed for Large Volume Phlebotomy (LVP) in Black Rhino

- Redon vacuum drainage system
    - Privac 600 ml, OK-model, hose 125 cm, Large Lock Connector
    - Vacuum 0.9-1 bar
    - Hoose-needle connector
    - Male luer lock ring x 1.8 inch hose barb, Nylon, 25/pk

  NB: you will need bottles with a vacuum, bags don't work, because you only have access to vessels very low to the ground gravity won't help getting the blood out and the needle/hose will keep getting blocked by clotted blood because the blood flow is too slow!
- Needle
    - 1 inch 18G (Luer Lock)

  NB: > 1.5 inch does NOT work, the skin is only ca 0.6-0.7cm thick as well as the diameter of the vessel, so with a longer needle the bone/joint underneath will be hit which is painful for the animal!
- Additional: Cool spray (chloor-ethyl) (human pharmacy)

Use of chelators?

The long term treatment with chelating drugs is not proven to be effective and may even by contra-indicated, as the resorption of ions in the gut may be heavily compromised (e.g. copper). HBED chelators can be used orally [pers communication K. Sullivan, Disney, Florida USA], also deferoxamine has been used with success (Muneuchi, 2018). Natural chelators like tannins seem to be effective (branches, leaves).

Appendix 6: List of laboratories

Test		Sample type	Laboratory	Contact details
Iron values		Serum	UVDL, Veterinary Faculty, Utrecht University, the Netherlands	j.vossen@uu.nl
Progesterone		Faeces	Chester Zoo, UK	j.ohanlon@chesterzoo.org
		Faeces	Dept. of Biomedical Sciences - Endocrinology University of Veterinary Medicine Vienna, Austria	Franz.Schwarzenberger@vetmeduni.ac.at
		Serum	Rotterdam Zoo, the Netherlands	l.van.sonsbeek@diergaardeblijdorp.nl
TBC	Interferon Gamma Test	Fresh heparin whole blood	Veterinary Faculty Utrecht, the Netherlands	v.rutten@uu.nl
	PCR and mycobacteria culture	BAL, any suspicious (organ) material	National reference lab: Depended on country, good results with German national TB reference lab in Jena and Dutch national reference lab in Lelystad	Germany: Stefanie.Barth@fli.de The Netherlands: ad.koets@wur.nl
	Serology			