Final Thesis

Neonatal mortality in blackbuck antelopes at Kolmården Zoo

Adam Sandberg

LiTH-IFM-Ex--xx/xxx--SE

Contents

1 Abstract

The neonatal mortality in blackbuck fawns at Kolmården Zoo has been a problem for several years. To increase the chance of survival the fawns have traditionally been born indoor during winter in order for them to be old enough to handle the stress in the mixed species exhibit. Despite precautions the mortality was still high. A study was carried out to compare success between indoor and outdoor births and also to map the suckling behaviour, with focus on suckling duration and suckling bouts, and discern whether any behavioural abnormalities existed. Gamma values were calculated from a spectral density function to localize patterns in the suckling behaviour. Eight does and their newborn fawns were used in the study. Four does were placed in an indoor pen while the other four remained with the herd in the exhibit. The results showed a possible case of behavioural abnormality and that the suckling duration did not match data from the literature. The mean value for does in the indoor pen was 38,9±38,3 s, and the median was 28,2 s. The outdoor fawns contributed with too little data for calculations. Since this study failed to contribute with enough data to draw conclusions regarding the mortality, further studies are needed to determine the cause/-s for the high fawn mortality.

Keywords: blackbuck, suckling behaviour, mortality, time series analysis

2 Introduction

The Indian blackbuck (*Antilope cervicapra* Linnaeus, 1758) is a herd living antelope of the Indian and Pakistan grasslands. It was formerly one of the most common ungulates in Pakistan and India numbering almost 4 million in the beginning of the 20th century, but due to intensive hunting and habitat destruction it is now rare. In the 19th century herds as large as 8000-10000 animals occasionally congregated. In 1947 The Wild Life Preservation of India estimated 80000 blackbucks, but only 8000 remained in 1964. In 1977 unofficial counts said 5000-10500. In Pakistan the situation was even more desperate with no more than a few blackbucks left in 1970 (Mungall 1978). In 1989, however, the population in India was estimated to 43500 individuals (Ranjitsinh 1989) and the species is now classified as CITES III (CITES 2008). There are currently 2017 blackbucks in captivity (ISIS 2008), however the species also exists as “exotic game” on ranches in e.g. Texas, US. Further information on the species can be found in Appendix I.

The blackbuck is noted for its ability to reproduce very well in captivity (Mungall 1978), and since the species is threatened it is desirable for e.g. a zoo to have a stock that replenishes itself and grows. That was not the case at Kolmården Zoo were the blackbuck herd had been substantially reduced in the decade preceding this study, and in addition the neonatal mortality had been elevated for several years prior 2007[1]. In the light of these trends it was determined that a study would be carried out to determine what caused the mortality issues among the blackbucks.

2.1 Behavioural abnormalities **Strukturera om något**

Neonatal death may be a sign of behavioural abnormalities in the mother that influence her ability to care for her young. Abnormalities might occur due to problems during early life, social or other stress during parturition or the pre-/neonatal period. Patin *et al.* (2002) states that prenatal stress affects several maternal behaviours in rats crucial to mother-pup recognition and pup defecation/urination. Social stress from being housed in a small area crowded with other individuals may thus negatively affect the does´ maternal abilities.

In nature, blackbucks, like many other antelope and deer species, give birth in isolation, and only visit the fawn a few times per day to suckle it. Hence, the blackbuck is a typical "hider" and Mungall (1978) claims that the doe prefers to keep conspecifics at least 10 m away from the newborn fawn and will even run off intruders with an aggressive, so called head-up display. It is likely that keeping the pregnant does in the small indoor space with many conspecifics and with nowhere for the neonates to hide, might have disturbed key maternal behaviours. Studies in rodents suggest that stress during gestation alters postpartum maternal care (Champagne & Meaney 2006 cited by von Borell *et al.* 2007), which might affect the most important contributing factor to a fawn's survival, i.e. the intake of nourishment. Furthermore, evidence has been provided that maternal psychosocial stress in humans reduces the length of gestation and concomitantly the weight at birth (Merlot *et al.* 2008), and Guo *et al.* (1993) states that stress during the first half of gestation in rats, induced small for date, i.e. smaller than the mean for that age, offspring of which 74.4 % died during the peripubertal period.

2.2 Trauma

Trauma caused by attacks from one or several of the other species kept in the Savannah exhibit of Kolmården Zoo is not uncommon. Notorious species like Grevy´s zebra are kept there, and also other antelopes, giraffes, deer and ostriches. Attacks on neonates are not uncommon and even if such

an attack does not result in immediate death, it will greatly reduce the fawns´ chances for survival.

2.3 Hypothermia

Hypothermia may also be a contributing factor to increased neonatal mortality, especially if the fawns suffer decreased birth weight due to stressed mothers as described in rats by Cabrera *et al.* (1999 cited by Kaiser & Sachser 2005) and in humans (Merlot *et al.* 2008, Homer *et al.* 1990). In Kolmården the fawns have traditionally been born indoor during the winter, in order for them to be agile enough in time for the summer season where they will have to cope with a mixed-species situation in the exhibit. Although the stable is heated and the indoor temperature is set to +15 ^{o}C there may be large differences between floor and roof, and during very cold spells the temperature at floor level may go down considerably below +15 ^{o}C. If born outdoor, even in the summer the Swedish climate may be too harsh for a tropical species like the blackbuck if not provided with ground vegetation that can create warmer micro-climate it might succumb. KAN inte smälta maten. Dehydrering, yttemp. Hur relevant var det?

2.4 ARBs *Ta upp helt kort i Behaviour abnormalities istället?*

Animals in zoos do not infrequently show overgrooming, self-biting, the eating of inedible objects (‘pica’), rhythmic rocking or pacing, and other ARBs (Abnormal Repetitive Behaviours) (Mason *et al.* 2007). The conduction of such sequences of behaviour is unfortunate because of the divergence from the natural repertoire which in turn might question the animal’s suitability for reintroduction. The occurrence of ARB might even indicate C.N.S dysfunction (Mason *et al.* 2007). Mason *et al.* (1995, cited by Garner 2005) states that stereotypies might lead to impaired growth in offspring, or even elevated offspring mortality (Sørensen and Randrup 1986, cited by Garner 2005). Hence this study also aimed at finding out if ARBs occurred in connection with the pregnant females.

2.5 Inbreeding

Inbreeding may have important fitness costs and is likely to shape life-history traits in all living species (Charpentier *et al.* 2007), induce inability to integrate behaviour into a coherent pattern (Wainwright 1981) and reduce growth in neonates (Carolino *et al.* 2004). It is also known to decrease survival and reproduction in captive populations (Jiménez *et al.* 1994), as reported in captive wolf (*Canis lupus*) by Laikre & Ryman (1991). Cassinello (2005) showed in a study with three species of gazelle that in females the longevity is negatively associated with inbreeding coefficient.

The same study also showed, in one of the included species, a significantly negative relationship between mothers inbreeding coefficient and sons and daughters longevity.

When inbreeding is concerned, it has not been possible to trace back the origin of the first blackbucks arriving at the zoo in the 1960s. There were no functional breeding programmes in those days and the animals were allowed to mate as they pleased. Since the blackbucks were kept both in the Safari park and in the zoo, there was a routine to keep a sterilized male in the zoo exhibit during the summer, and then to replace him by a fertile one in the late summer/early fall to time the births to the winter stable season. In the Safari park several males were kept together with the females in the summer so that the fawns would be born in the winter. This in combination with the does giving birth very close in time in the same stable compartments, made it impossible to identify the fawns' parents as the herd grew. Very few unrelated animals were imported and without any effective programme to manage the genetic diversity, the blackbuck stock at Kolmården Zoo is now most likely highly inbred.

2.6 Aims of the study

1. Make a thorough analysis of the studbook to reveal fawn mortality in the long term, and attempt a hypothetical inbreeding coefficient.
2. Observe behaviour crucial to fawn survival i.e. suckling behaviour.
3. Record abnormal behaviours in the does.
4. Record inter-specific behaviours which might affect fawn survival.
5. Record body surface temperature as an indirect measure of hypothermia in the fawn.

3 Materials and methods

The study was carried out at Kolmården Zoo, 30 km north-east of Norrköping, Sweden.

The studbook analysis of long-term mortality included all births and neonatal deaths (<30 days of age, zoo standard) from 1990-2007. With the current software (ARKS 4.0) it was not possible to extract the necessary data automatically, and therefore the extraction was made manually from print-outs. Furthermore, a hypothetical inbreeding coefficient was calculated by tracing the female ancestors of an arbitrary doe in today's herd back to the founders in the early 1970s. This was done as a worst case scenario, i.e. the mothers were as young as possible in order to produce as many generations as possible during the given time, and a best case scenario where the mothers were as old as possible.

3.1 Animals

Nine blackbuck (*Antilope cervicapra sp*) does had significant concentration of progesterone to be considered pregnant (Table 2), but only 8 were included in this study. Due to diffuse ID markings (notches in the ears), there was some uncertainty on the ID of the outdoor does (cf below) in relation to the fawns born.

Table 1. The age and designation of the subjects in the indoor pen and in the Savannah exhibit.

Does in the indoor pen	Does in the Savannah exhibit
V34: age 3	**V6**: age 6
V24: age 6	V7: age 4
V17: age 4	V15: age 2
V14: age 7	V36: age 6
	V33: age 6

3.2 Experimental setup

An analysis of the situation, based on the existing information in the studbook, in fawn autopsy reports, and interviewing the zoo keepers, pointed at several possible causes of the fawn mortality, with social stress in the indoor quarters in connection with deliveries, and hypothermia due to cold spells in the winter causing too low temperatures close to the pen floors being the most plausible. Hence the setup for the 2007 blackbuck reproduction was changed to address these factors:

1. Social stress due to crowding was reduced by allowing some does to give birth outdoor.
2. The risk of hypothermia was minimized by timing the deliveries to the summer.

Therefore, the pregnant does were divided into two groups: one with four does kept indoors in the Savannah stable, monitored by surveillance cameras, and the remaining four kept outside in the public display Savannah exhibit in the Safari park, observed with focal animal follow. This was the first time Safari blackbuck deliveries were timed to the summer and allowed to take place outdoor[1].

3.3 Climate factors

A log of temperatures, humidity and wind velocity was obtained from the Swedish Meteorological and Hydrological Institute for the period of the births of the Savannah exhibit fawns. These data came from the nearest weather station, which was located approximately 10 km from the zoo. The optimal approach would have been to measure parameters in the direct vicinity of the fawn, but this was not practically possible.

[1]pers. comm., Bengt Röken, Kolmården Zoo

3.4 Data analysis

An analysis of time series (Chatfield 1996, chapters 6 & 7) was also conducted for suckling duration and clustering. Gamma values were calculated from a spectral density function which is the Fourier transform of the time series.

The gamma value indicates how conservative the subjects are when performing a behaviour i.e. aggregation and duration of the performed behaviour. Spectral analysis is usually made on time series to analyze the frequency components in a series. By Fourier transform any time series can by transformed to a sum of sine curves, the sine curves are specific in their frequencies and amplitudes. Hence the amplitudes of the frequencies indicate their importance in the time series. If lower frequencies have the largest amplitudes they dominate the time series and then there is a high degree of autocorrelation in the series. By making a linear regression on the amplitudes of the frequencies (in the log log plane) one can use the inclination of the slope to test if the low or high frequencies dominate the time series. A positive slope indicates autocorrelation and hence a conservative behaviour. The test was conducted in order to investigate if there was a pattern in the suckling behaviour. The spectral analysis was done over the whole time series as well as over specific periods since the data was not linear over the whole time time series.

3.3 Housing and management

3.3.1 The indoor pen

Four does were moved to a 4 by 6m indoor pen (fig 1) a few days *pre-partum*. The pen had 2m high wooden plank walls, but was open at the top; the stable outer roof was 6m above the floor. Slits in the pen's walls towards the keeper area were covered to reduce possible startling effects or stress from keepers and visitors passing by. The floor was covered with a 10 cm layer of straw and the pen was divided longitudinally by a soft fabric curtain (Viraduk®) supplemented with a row of straw cubes on the floor, which allowed the animals to hide and get out of sight of each other. The animals were left undisturbed except when keepers brought food and water. The animals were under continuous observation by surveillance cameras from the day before the predicted birth until three days (the critical period for survival[2], fig?) after the last doe gave birth.

[2]pers. comm., Bengt Röken, Kolmården Zoo

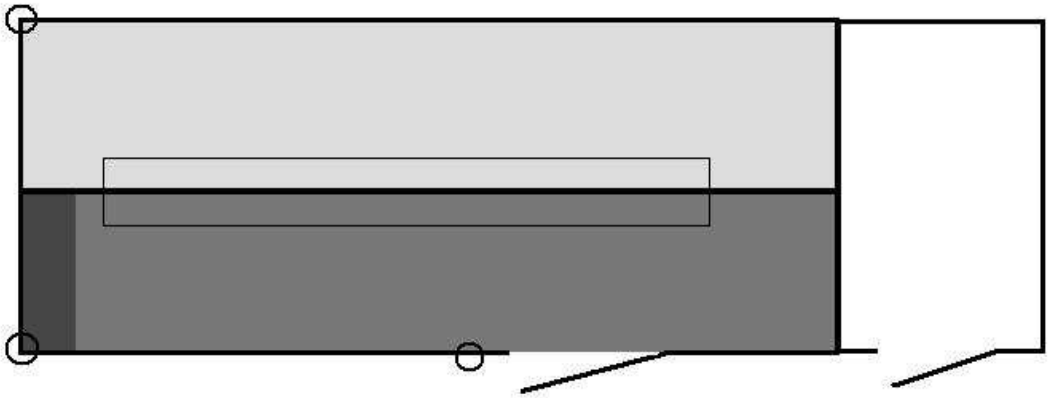


Fig 1. The indoor pen seen from above. Different shades of grey indicate coverage by the three cameras. The black line dividing the grey fields is the Viraduk® cover. The circles represent the cameras orientation in the pen and the square in the middle represents the straw cubes. The equipment was kept in the pen to the right.

3.3.2 The Savannah exhibit

The remaining four does (table 2) were left in the 6 ha Savannah exhibit (fig 2) together with the rest of the blackbuck herd totally consisting of 8 males and 17 females. In the exhibit resided several other species such as giraffes (*Giraffa camelopardalis)*, ostriches (*Struthio camelus*), brindled gnous (*Connochaetes taurinus*), Grevy's zebras (*Equus grevyi*), elands (*Taurotragus oryx*) and chitals (*Axis axis*). During the observation period there were also lots of visitors driving their cars through the exhibit. The dashed lines indicate the outer fence of the exhibit. Black lines indicate otherwise restricted areas for the animals except juvenile i.e. smaller individuals.

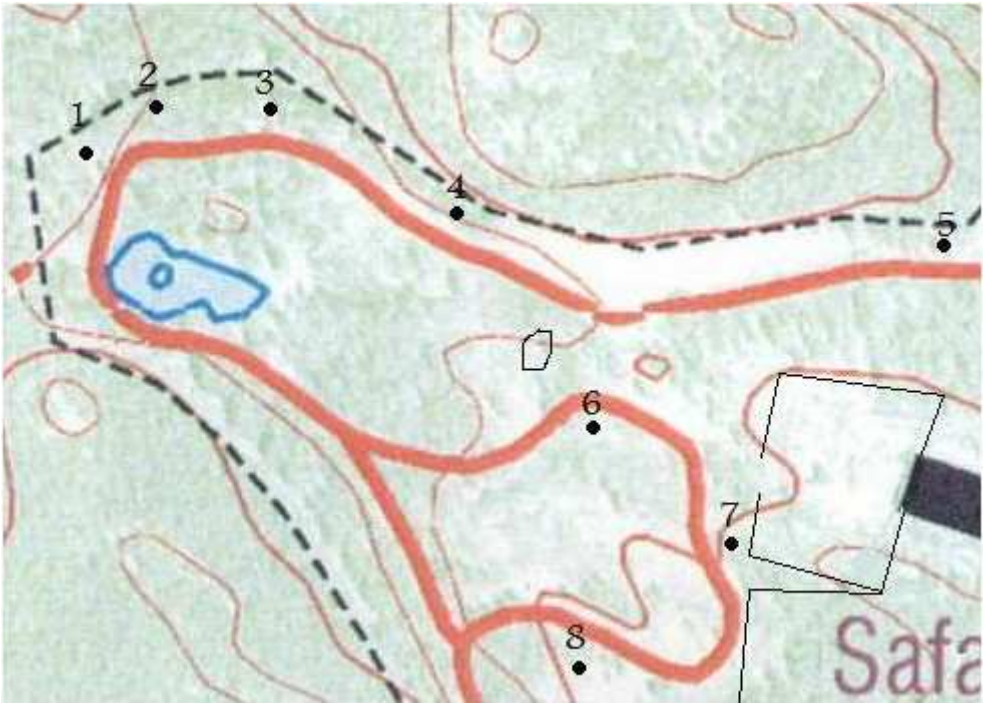


Fig 2. A map over the Savannah exhibit. The dashed lines indicate the outer fence and the black lines indicate the inner fence or, for larger and adult animals, otherwise restricted areas. The numbered black dots indicate the lying out positions used during the study. The bold red line indicates the main road.

3.4 Behavioural observations

The approach was to record behaviours believed to be critical to the fawn's survival and to compare the behaviour of the two groups of does. The most important parameters measured were the duration of suckling and suckling bouts, the number of sucklings in a bout and the duration of resting intervals. The observed behaviours can be seen in table 3.

Table 2. Ethogram of suckling related behaviours

Behaviour	Definition
Suckle	Attach to teat
Cease suckling	Let go of teat

3.4.1 Individual recognition

Even to a trained eye, blackbucks are difficult to recognize individually, so marking the pregnant does was vital for viable observations.

Two does in the Savannah exhibit were immobilized and equipped with dog collars in different bright colours. Unfortunately, the pregnancies were deemed to be in a too advanced stage to safely immobilize the remaining does. Due to this, and the in some cases diffuse ear marking, the identity of the mothers is uncertain.

Tagging with dog collars could also have been utilized in the marking of the indoor group, but since the cameras were only black and white (inevitable with IR-light at night) it was not possible to separate different colours, and black and white patterns would not be resolved by the cameras. In the indoor pen the back fur of three of the does was shaved on different locations, while the fourth was equipped with a dog collar, in connection with them being immobilized and moved into the pen.
Marking the fawns was not advisable due to the stress it might cause.

3.4.2 The indoor pen

The behaviour of the animals in the indoor pen was recorded by 3 video cameras and stored on the hard drive of a DVD in 4 h segments and then analysed in Observer XT (Noldus Information Technology, Holland). A video splitter was used to cyclically shift between cameras. Each camera was on for 2 s, resulting in 4 s gaps on each camera, which lead to some uncertainty in the logging of some behaviours and the identification of the fawns. This setup was chosen instead of having 3 continuous, smaller frames for each camera on the screen, because limited picture resolution would have made animal identification difficult. The recordings were then analysed in Observer XT (Noldus Information Technology, Holland). After the burning to DVDs the video files showed a gap of 20 min before the next 4 hours of video began. Due to this problem each file is only 3 h 40 min long and 20 min observation time is lost on each file. This of course influenced the results. The distribution of the total observation time can be found in table 4.

3.4.3 The Savannah exhibit

The animals on the Savannah were observed on-line using focal follow sampling (Altmann 1974) on a Psion Workabout Palmtop computer operating the custom made version of the Observer 3.0 (Noldus Information Technology, Holland). The data was transferred to a PC for later analysis in The Observer 3.0. The distribution of the total observation time can be found in table 3.

Table 3. The distribution of the total observation time for the indoor pen and the Savannah exhibit.

Date	Fawn #	Indoor pen	Comments	Savannah exhibit	Fawn #	Comments
13		24 h				
14	1	24 h		~1 h	1	Computer failure
15	2	24 h		~8-10 h		
16	3	24 h		~8-10 h		
17		24 h				
18	4	24 h		~1 h	2	Fawn abandoned
19		24 h		0 h		Fawn injured --> put to death
20		24 h				
21						
22						
23						
24				0 h	2	(2) Born late at night
25				~4 h	4	(2) Fawn badly injured
26				~8 h		
27				~8-10 h		(4) Last day of critical period

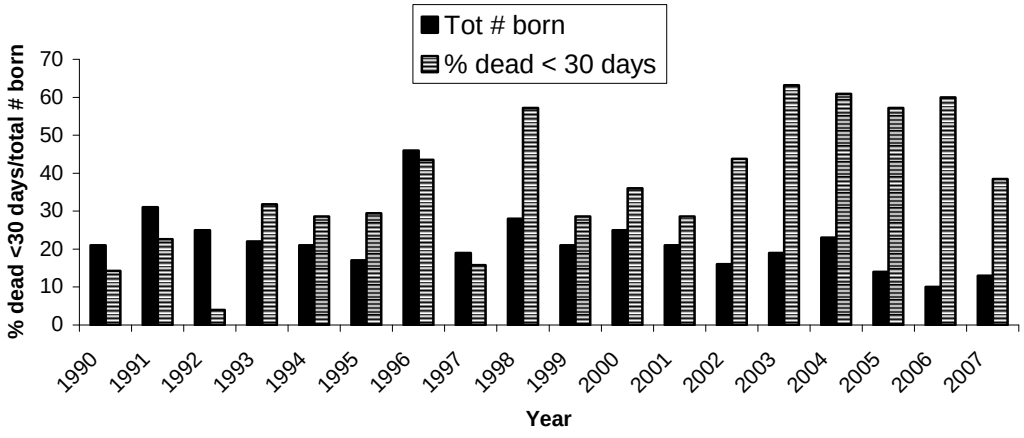
4 Results

4.1 Studbook evaluation

4.1.1 Neonatal mortality

Fig 3 shows the development of neonatal mortality from 1990 to 2007. Neonatal mortality was slightly higher a few years prior 2007.

Fig 3.The fig shows the neonatal mortality (<30 days old) for the blackbuck antelopes at Kolmården Zoo from 1990-2007.



In fig 4 all neonatal deaths from 1990 to 2007 are included and sorted after age at death, where most deaths are gathered within 4 days of age.

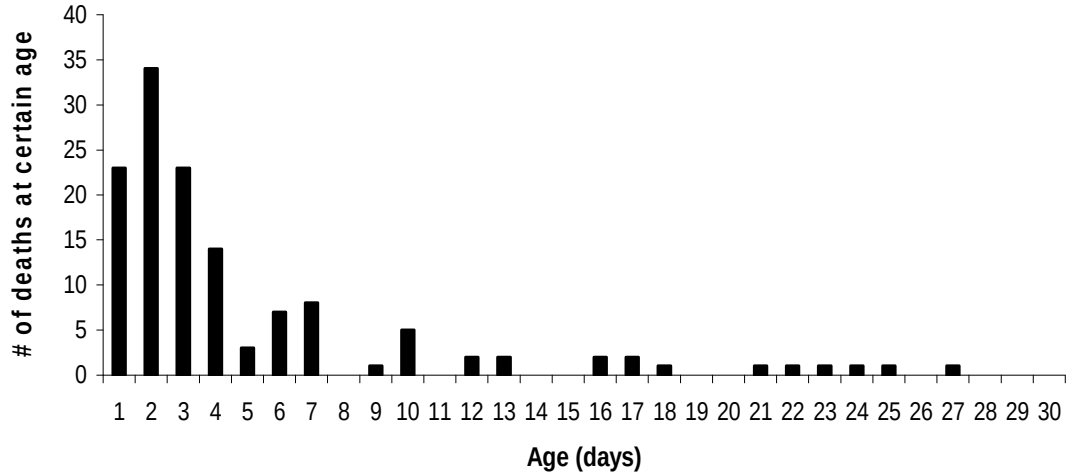


Fig 4. Number of neonates that died <30 days of age during 1990 and 2007, sorted by age at death.

In order to determine if season i.e. summer vs. winter was a factor that affect the neonatal mortality, season was set up against neonatal mortality. Season did not appear to affect mortality to any larger extent.

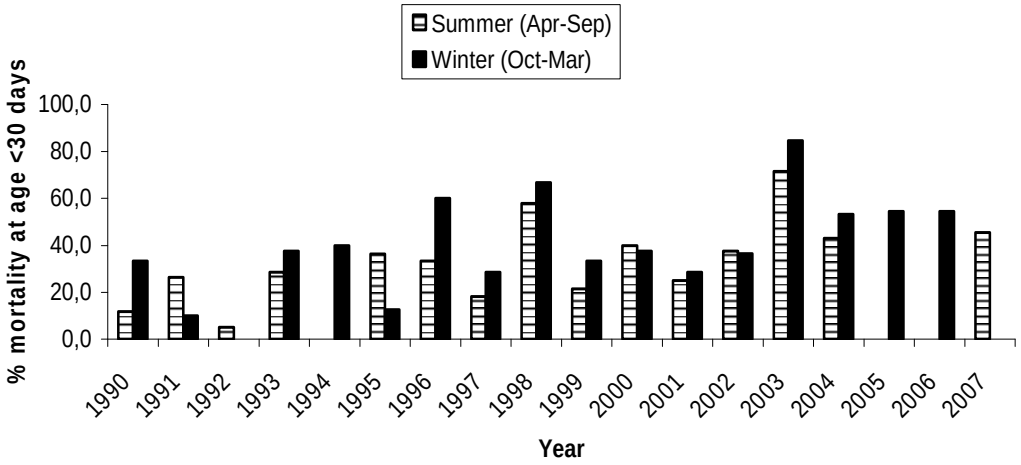


Fig 5. The percentage of fawns born that died <30 days of age from 1990 to 2007.

4.1.2 Population dynamics

The studbook was evaluated from the start of the Kolmarden blackbuck stock in the late 1960s until present time to reveal how certain events, e.g. the fusing of two sub-populations in a single enclosure, affected the age – and gender structure and the total size of the stock. Between 1997 and 2007, the stock was reduced in size by more than 50 %.

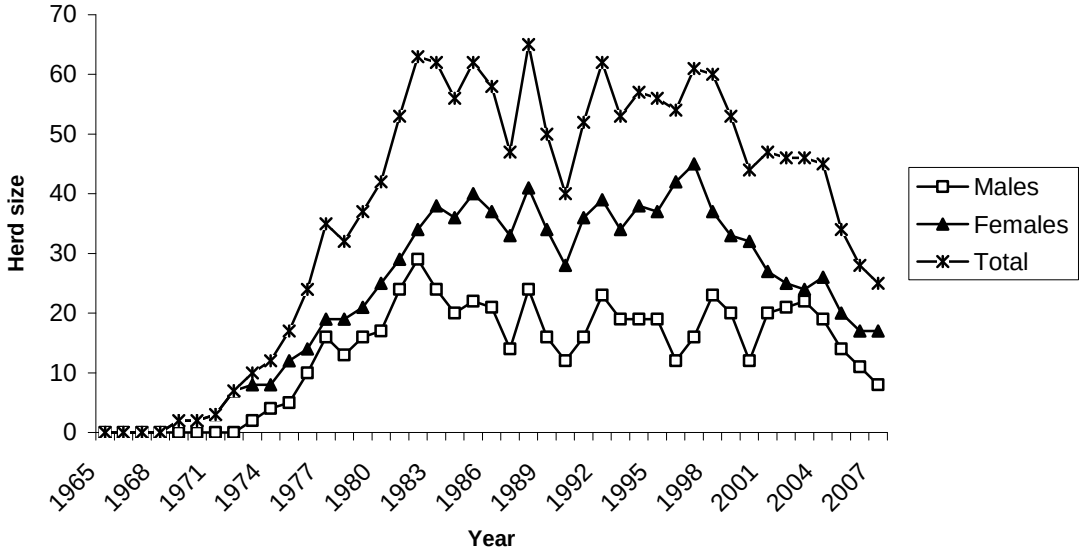


Fig 5. The population dynamics of the Kolmården blackbuck stock from 1965 to 2007.

4.1.3 Current age structure

An age pyramid was also constructed based on the current (2007) age structure of the herd. Unfortunately, with the current software (ARKS 4.0), it was not possible to include the age structure of former years.

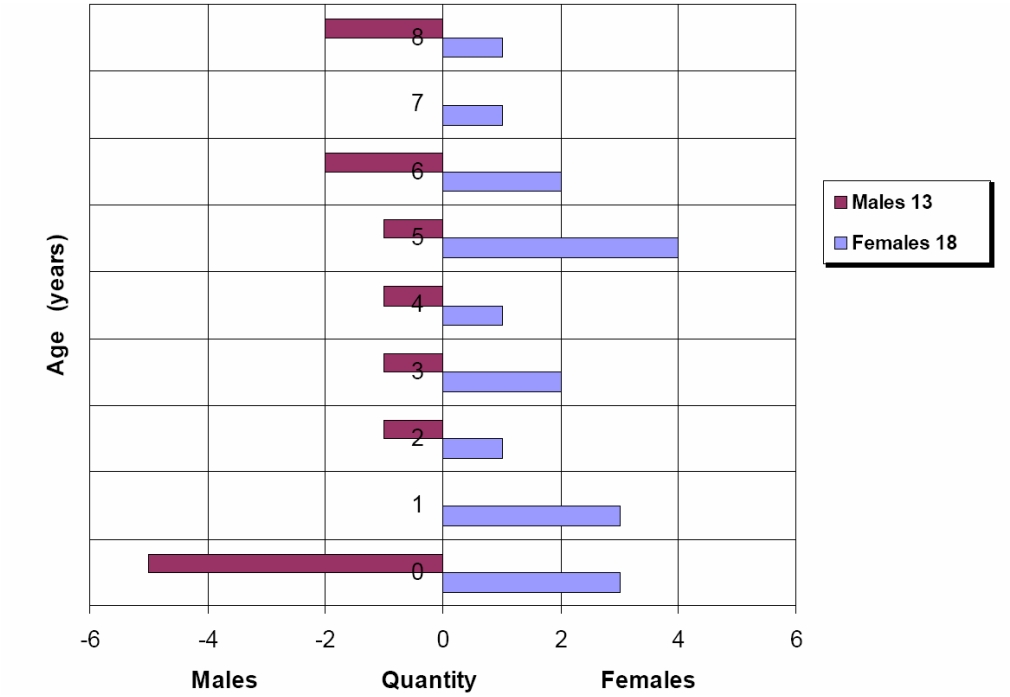


Fig 6. The current age structure of the herd at Kolmården Zoo.

4.2 Births and deaths during the test period

4.2.1 The indoor pen

Four fawns were born in the indoor pen and all of them survived the first period of three days. One fawn (no 4, table 4), however, died on its sixth day of life. The death was caused by an infection.

4.2.2 The Savannah exhibit

Five fawns were born in the Savannah exhibit and three of them survived the first period of three days. One fawn (no 2, table 4) was put down due to a fractured carpal joint. The fracture might have been an accident, although a visitor claimed that she saw a chital buck attacking the fawn.

4.2.3 Climate factors

A second fawn (no 1, table 4) died on its 6th day of life due to hypothermia according to the *post mortem*. The temperatures during the test period are shown as two means per day, 6 a.m. to 6 p.m., and 6 p.m. to 6 a.m., while the humidity and precipitation is shown as one daily mean. The precipitation is measured in mm on the temperature scale. The numbers represent the fawns (table 5), the asterisks represent births and crosses deaths.

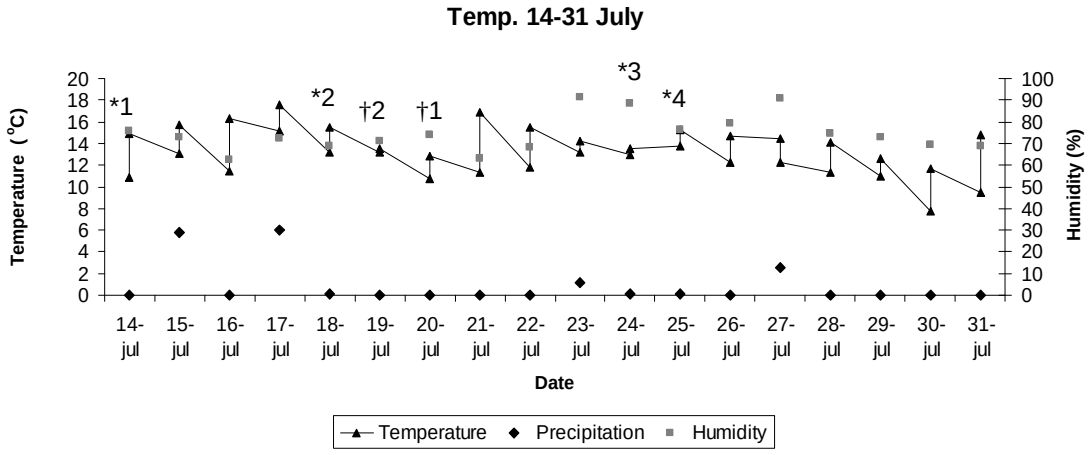


Fig 7. The graph shows variations in temperature, humidity and precipitation during the test period 14th to 31st July. The numbers represents the fawns in table 5, an asterisk represents births and a cross represents deaths.

The fawn was found dead on the 20th July and was thought to be the fawn born on the 14th of July (no 1, table 5). This fawn weighed less than

2.5 kg which is at least 0.5 kg below average (Mungall 1978). The *post mortem* showed an empty abomasum, an almost empty colon with no meconium left and a heart haemorrhage.

The mortality during the test period in 2007 was thus 3 out of 8.

4.3 Suckling behaviour

4.3.1 The indoor pen fawns

The DVD-recorded observations in the indoor pen lasted for eight consecutive days, from the 13th to the 20th of July, around the clock, which covered the critical period (3 days) of all four fawns.

The following graph (fig 5) shows the distribution of suckling durations in the indoor pen with four fawns and three does included. The doe designated V24 (table 1) did not allow any fawn to suckle her and is excluded from suckling calculations. The remaining does are separated in the figure.

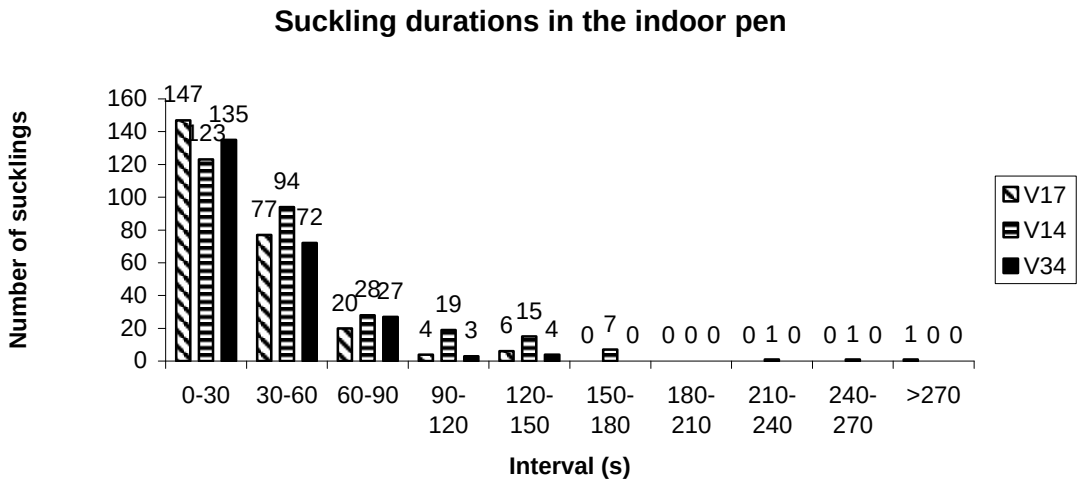


Fig 8. Distribution of suckling durations in the indoor pen with the does separated.

Table 4 shows mean value, SD and median for suckling duration in the indoor pen does.

Table 4. The mean with SD and median values for suckling time for the does in the indoor pen.

Dam	Mean+SD	Median
V17	33.6±41.4 s	23.7 s
V14	48.0±42.3 s	35.0 s
V34	33.7±26.1 s	26.2 s
V17+V14+V34	38,9±38,3 s	28,2 s

A two-tailed t-test showed no significant difference in suckling time between the three does in the indoor pen.

In fig 6 all sucklings in all intervals are sorted, summed and shown as a percentage of the total suckling time. The total suckling time in the indoor pen was 29997 s (total observation time was 192 h).

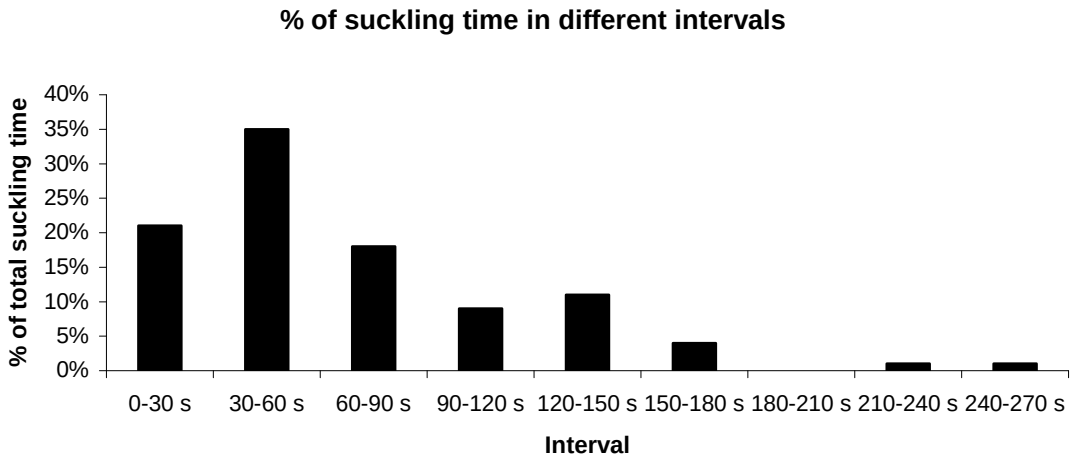


Fig 9. The figure shows the distribution of suckling time in all intervals for the does in the indoor pen.

4.3.2 Suckling bout

When suckling bout was calculated the principle was to measure the total duration of one to several sucklings between two "long" periods of rest. A long period of rest was settled to be 15 minutes. Such a suckling bout was considered to be one "meal" and usually contained several sucklings. Unfortunately this measure was flawed because of the particularly frequent occurrence of allosuckling. Only the does in the indoor pen were included since only they provided sufficient data for the calculations.

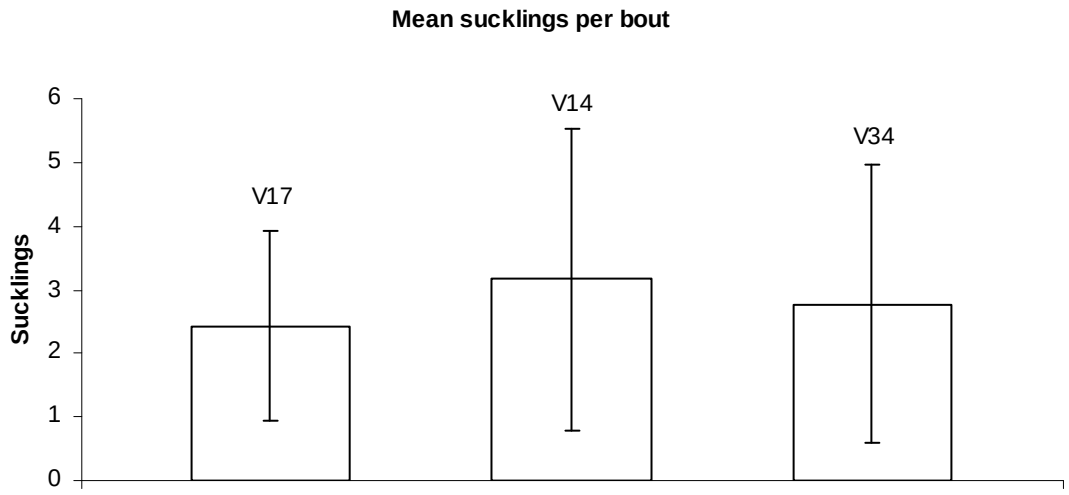


Fig 10. The graph shows the mean number of sucklings in a bout for the three does in the indoor pen. SD is indicated by vertical lines.

The mean number of sucklings in a bout for V17, V14 and V34 was 2.4±1.5, 3.2±2.4 and 2.8±2.2 respectively (fig 9).

A mean value of the suckling bout duration was calculated and the results are shown in fig 10.

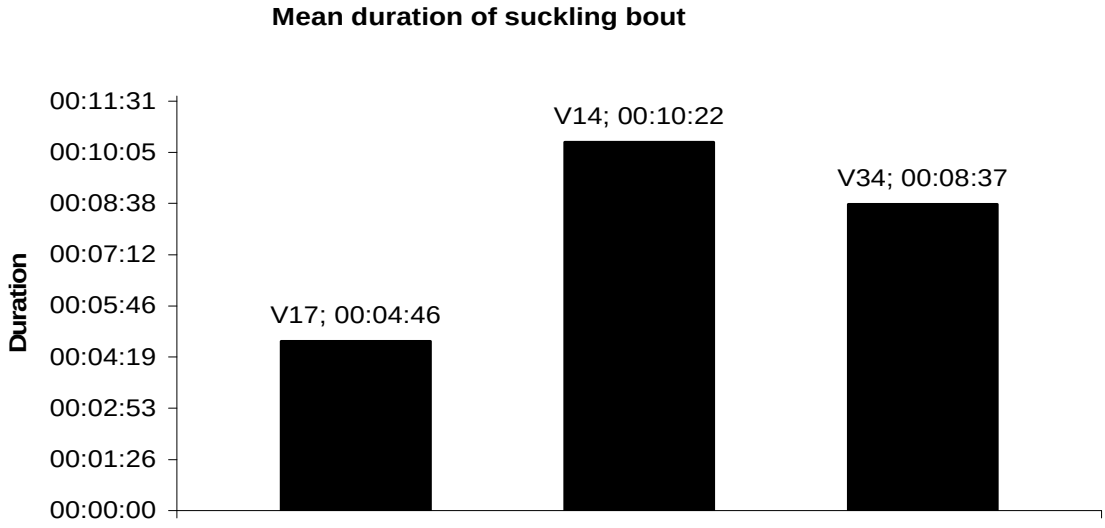


Fig 11. Graph shows the mean duration of a suckling bout for the three does in the indoor pen.

4.3.2 The Savannah exhibit fawns

The outdoor observations lasted from the 14th to the 27th of July. Due to the lack of sufficient number of observers it was not possible to cover 24 h a day or the whole critical period for each fawn in the Savannah exhibit. The doe designated V6 are excluded since her fawn got put down due to serious injury.

Fig 12 shows the distribution of suckling durations in the Savannah exhibit.

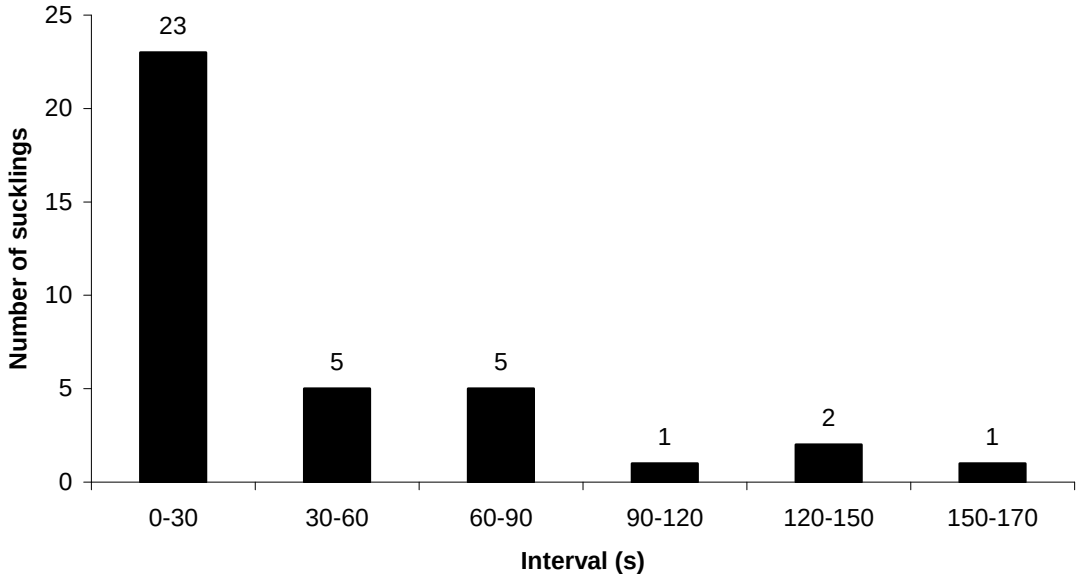


Fig 12. Distribution of suckling durations on the Savannah. n=37. Three does included.

The mean suckling duration for the Savannah exhibit does, with all three does compiled, was 40.3±42.4 s. The median was 21.6 s.

The individual designated V33 contributed with 70 % of the data from the Savannah and therefore only that particular doe is presented individually. Fig 13 shows the distribution of its suckling.

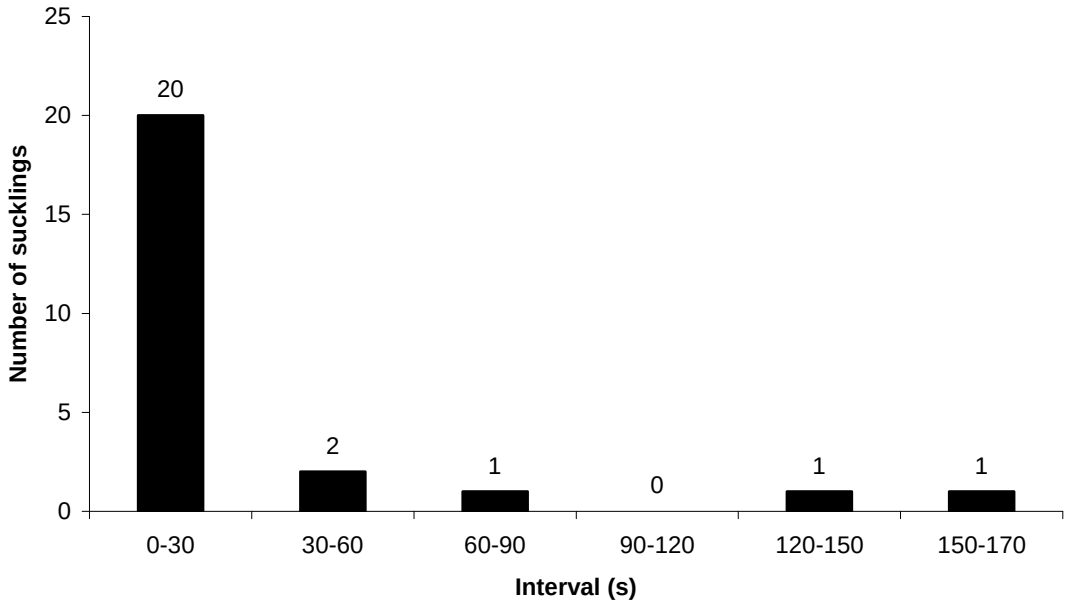


Fig 13. Distribution of suckling durations for V33. n=25

The mean suckling duration for V33 was 40.2±32.5 s and the median was 29.2 s.

4.4 Abnormal behaviours

4.4.1 The indoor pen

A slightly unforeseen behaviour in the indoor pen was allosuckling, a behaviour where neonates suckle milk from females that are not their genetic mother (Roulin & Heeb 1999). Since the fawns were not marked the allosuckling was detected when two fawns, at several occasions, suckled the same doe at the same time.

The doe designated V24 paced back and forth in the indoor pen during the entire test period. She did not allow any fawn, even her own, to suckle and even thwarted attempts with force.

4.4.2 The Savannah exhibit

During the test period no abnormalities could be seen in the conduction of the suckling behaviour, and all elements of the behaviour were conducted except for the vocal summon which was seldom heard from the doe. A vocal response from the fawn was never heard.

4.5 Inter-specific interactions

The fawn in fig 14 was probably assaulted by one of the zebras[3] and the left side of its face is badly battered resulting in a swollen eye and lower jaw. It

was also lame, and had a slightly deformed left ear. In spite of these injuries, this fawn survived the three day test period.

Fig 14. A blackbuck fawn with a swollen left side of the head and deformed ear.

On one occasion a chital doe disturbed a lying fawn and caused it to run away. That incident was the only verified observation of another species disturbing a lying fawn.

At one point a doe and her fawn found themselves in the middle of a small herd of eland antelopes (*Taurotragus oryx*) where the doe eventually completed the suckling bout and left her fawn for it to find a new resting place. The elands, however, seemed intrigued by the fawn, and did not leave it alone long enough for it to find a new hiding place.
After approximately 5 minutes the fawn was forced to leave the immediate vicinity of the elands, and eventually found a new resting place on higher ground 10 m away from the elands.

4.6 Spectral analysis of suckling frequencies and durations

This analysis gives a number of gamma values where gamma represents the slope of a curve. Gamma is the notation of the whole curve, gamma first is the first half of the curve, gamma start is the first ten values and gamma last is the last half of the curve. The two tables show values calculated for the suckling durations and resting periods between sucklings. A gamma value of ~2 is considered high and would, in this case, indicate that the behaviour is conservative i.e. the probability is high that the behaviour is aggregated and performed in the same manner next time. A negative gamma value indicates that the behaviour is not aggregated and is not performed in the same manner next time it is performed.

Table 5. The values calculated for the suckling duration for the three does in the indoor pen.

Suckling	V17	V14	V34
Gamma	0.2756	0.2046	0.0172
Gamma first	0.3359	0.4614	0.0517
Gamma last	-0.3206	-0.0083	0.0111
Gamma start	1.1167	0.7368	0.3699

As can be seen in table 5 most values are positive but few are above 1. This indicates that suckling behaviour is somewhat conservative but also could be performed in a random way .

Table 6. The values calculated for the resting periods between sucklings.

Not suckling	V17	V14	V34
Gamma	-0.2838	-0.0458	-0.0073
Gamma first	-0.0345	-0.0318	0.1448
Gamma last	-1.7278	-0.3351	-0.1701
Gamma start	0.9795	-0.1938	1.38

As can be seen in table 6 most values are negative but few are even below -1. Some are even positive. Most probably the duration of resting periods often differs.

4.7 Lying out positions

The numbered black dots in fig 2 indicate lying out positions used during the study by fawns in the Savannah exhibit. In order to facilitate survival among the fawns it might prove important to assess what parts of the exhibit are used for lying out. It might also prove useful to investigate what kind of ground texture that is preferred. Therefore, pictures of the eight positions used can be found in Appendix II.

5 Discussion

5.1 Studbook analysis

The analysis showed that the population was reduced with more than 50 % during 1997 and 2007. The male part had been fairly stable until 2004, whilst the female part has been declining rapidly since 1997. The reason for this may be that two sub-populations were fused in 2004, which might require some time for adaptation to different habitat and perhaps a more crowded situation. The neonatal mortality increased after 2003 for uncertain reasons, although a plausible explanation is that accumulating inbreeding defects reduces the fawning success. Very few animals were used at the start-up of the herd, and the sons of the breeding buck usually took over after his demise. Over the years a minority of imported bucks have been used as breeding males. The records show only three buck

importations with uncertain genetic relationship to the residents of Kolmården.

The most common causes for neonatal death, extracted from *post mortem*-reports, were emaciation, abandonment, not fed and hypothermia. However, the course of events leading to a fawns death may be too complex to assess without actually observing the process. Abandonment, for example, may just indicate that the mother is primiparous and lack experience. If the fawn is not fed do not necessarily point at lack of maternal care, but only that the fawn was not able to stand and hence could not reach a teat. It is thus difficult to draw conclusions from this analysis.

5.2 Mortality

This study aimed at investigating the possible causes of neonatal mortality among captive blackbucks at Kolmården Zoo. During the study 3 out of 8 fawns died due to three different causes.

One fawn died due to an infection, one from hypothermia, and one had to be put down due to a fractured carpal joint. Hence the possibility to test several causes of death was limited. However, in an attempt to find ways of improving the survival rate, the delivery setup was different in 2007 compared with previous years: the indoor pregnant does kept together were few in relation to previous years and they were separated from the rest of the herd, and outdoor deliveries were approved for the first time in the Savannah exhibit. These changes addressed two possible factors involved in the mortality: social stress and absence of doe-fawn privacy. Even though only four does were kept together in a relatively spacious compartment, one doe behaved in a way that indicated stress: She paced back and forth during the entire test period for no apparent reason. She was not attacked or else bothered by the other does and the keepers had never experienced such stress among the animals before. She was the last one to give birth, and she did not show any interest in her fawn. It might have been because she was unable to find enough privacy in connection with delivery. In the future, does behaving stressed should be offered the possibility to get further away from the others, and maybe even be moved to a separate compartment.

Another possibility may be that a primiparous doe would lack the necessary experience to be able to care for her young (Mungall 1978). However, V24 was six years old and, at this age, she should have been able to carry approximately 3 fawns prior to this one and hence should have the proper experience to be able to care for yet another one. Unfortunately, no records on her possible previous births could be found in Kolmården´s studbook due to the difficulty to observe such events, but most likely this fawn was

not her first. However, one must consider that the effects stress during gestation depends on the nature of the stressor/-s, age of the subjects and species. Fonseca *et al*. (2002) describes the effects of stress in laboratory mice: *The effects of maternal stress on gestational data and reproductive parameters are known to vary as a function of the type of stressor, the age and strain of the experimental subjects used and, among others, the nature of the dependent variables evaluated.* One should also consider, as pointed out by Kaiser & Sachser (2005), that maternal effects on neonatal behaviour might be a way for the mother to increase her own fitness, by e.g. decrease the offspring's aggressive behaviour in crowded situations. This factor might prove important, especially if the fawn is born under certain conditions, i.e. the indoor pen, and soon after birth transferred to a habitat with other conditions i.e. the Savannah exhibit.

There were no indications of behavioural abnormalities affecting the mortality that might be correlated to inbreeding. On the contrary, inbreeding often makes animals more docile by reducing escape reactions like predator avoidance and increasing social tolerance.

Givskud Zoo in Danmark previously had a similar problem with approximately 90 % neonatal mortality. However, that number has dropped to about 10 % after establishing shelters in the enclosure[4] (see Appendix III). This option might be worth considering, should births continue occur outdoor.

5.5 Suckling behaviour

Suckling behaviour may seem a good way to measure energy intake i.e. the longer suckling time the more energy. According to Chaudhry & Tahir (1988) mean suckling time for a fawn during the first seven days of its life is 54 s and the range is 30-70 s. Mungall (1978) states that one to three minutes or even close to five is not an unusual suckling time during the fawns first days of life. In this study the suckling time was generally lower, and the range was much wider with suckling durations up to 270 s. This might be due to allosuckling with several fawns suckling and switching places during the camera cycling. However, some authors, e.g. Cameron *et al*. (1999) states that time spent suckling cannot be used to measure energy intake in horses (*Equus caballus*), and can therefore not be used to assess whether survival depends on suckling duration. Although, studying the suckling behaviour might yet give some indication…..

There was, for some reason, a drop out of 20 minutes in the video files (which were supposed to be 4 h). Due to this the logging of behaviours became even more uncertain and might influence the results. The spectral

analysis did not indicate whether the suckling behaviour is conservative or not.

5.3 Abnormal behaviours

Avhandlas i Mortality?

5.6 Allosuckling

With allosuckling performed, it turned out impossible to identify the fawns in the indoor pen. The assumption that the doe would care only for her own fawn was thus inaccurate. This complicated matters a great deal because without a single doe that cares for the fawn it is impossible to tell whether the death of a fawn is due to lack of attention or inability to perform key behaviours correctly. However, it is likely that the fawn that died due to an infection was delivered by V24, who did not suckle at all due to her restless behaviour. Hence she did not pass any colostrum to her fawn, and even if the other does suckled her fawn, they had no colostrum left for it. The rejected fawn was born two days after the rest and it is unlikely that any of the other does still had any colostrum. This line of reasoning is contradictory to the theory of allosuckling which claims, that the fawn might get an immunological benefit from suckling several does (Roulin & Heeb 1999).

[4]pers. comm. Kim Rasmussen, Givskud Zoo

Allosuckling may thus offer a fawn a chance to survive even though its mother rejected it. However, with only one day *post partum* of colostrum yield, this option would be very limited in blackbucks. As stated by Merlot *et al.* (2008) “The effects of maternal psychological stress on the innate immunity of the offspring are generally inhibitory.”
Allosuckling might be a negative burden for a doe in scarce food conditions in the wild. In captivity, however, it is hardly a disadvantage for does to nurse another fawn than their own, since they have access to a rather inexhaustible food source. However, plentiful food does not guarantee a doe enough resources to feed two fawns.

A very simple reason for allosuckling being accepted in this case might be that the doe sniffs the fawn to check its identity only after the suckling has already begun (Mungall 1978). Therefore another fawn may be allowed to suckle for a short time before the doe decides to reject it. With the high chance of repetition in a crowded pen situation, this fawn may be completely accepted by the doe. It cannot be excluded, however, that inbreeding also has led to an increased social tolerance in the does.

When crowded in a small area (~24 m^2) the does might have had some problem to discriminate between their own fawn and those of the other does. In the wild, the location of the fawn is an important factor in the recognition as well as the scent characteristics of the fawn (ref). With many fawns in close proximity these cues might be confused, leading to allosuckling being accepted or promoted.

In the Savannah exhibit allosuckling was not an issue. Obviously, when fawns are given the opportunity, they perform a more natural lying out and the first days almost only the mother meets her fawn unless spotted by other species like zebras or chitals. One allosuckling was, however, observed in the Savannah exhibit by an adolescent individual, which might indicate that it is not an entirely unnatural, only rarer, behaviour in the wild. Another plausible explanation is that the adolescent was an older offspring.

5.4 Inter-specific interactions

In the outdoor situation, a plausible explanation to attacks fawns is that there is no ground vegetation that could provide hiding or camouflage. A way to mitigate this would be to introduce some kind of artificial grass or bushes that could not be grazed nor destroyed in other ways. However, the attack on the fawn with the fracture may alternatively just indicate that the fawn was acting oddly and that this attracted the attention of the other animals. Artificial grass would of course prevent the visitors from seeing fawns at all times, but preserving the offspring should be considered of greater importance.

In the Safari park at Kolmården Zoo there is a closed public area called the Safari Camp, which is a genuine African tent camp offering lodging for smaller visitor groups. The camp is separated from the Savannah by only a simple wooden fence. A few times young blackbuck fawns were seen inside the camp area, although not during the test period, and were thus protected from other species´ assaults. There is a small enclosure on the Savannah made out of the exact same type of fence. In this small enclosure the grass is relatively tall from lack of grazing and would probably offer both shelter and more favourable micro-climate for young fawns. In this study, however, no fawn was observed using the small enclosure for its lying out. One possible reason might be that there are a lot of animals moving in close proximity to the enclosure, which is situated in a more central part of the exhibit than the Safari Camp. A more effective way to protect the fawn would be to build a similar fence to protect the more frequently used lying out positions shown in the introduction (fig 2) and Appendix II. If this sort of protection could be supplied in more

locations in the Savannah exhibit and readily be used by the fawns, it would offer significant protection.

5.5 Hypothermia, sätta ihop med mortality?

Artificial grass could provide a slightly more favourable micro climate and perhaps prevent a fawn to suffer from hypothermia. Although, adult blackbucks in Texas are known to endure temperatures well below -18 ^{o}C (Mungall 1978), neonates may still be vulnerable to the low temperatures that may occur during a normal Swedish summer. One fawn in the study died from hypothermia on its 6th day of life. It had no meconium left and its colon was almost empty. Probably, it had been taken care of to some extent and later abandoned. The loss of weight further strengthens that conclusion.

A thermal camera would seem to be a well suited tool to determine if a fawn is hypothermic. However, it is important not to induce stress by getting in close proximity to the fawn. In this study the attempt of getting pictures quickly had to be abandoned. One would simply have to get in too close proximity for the fawn to accept it. The summer of 2007 was rather sunny which also limited the quality of outdoor thermal images.

5.6 Conclusion Resurskonsult: 0730-720666

In conclusion, the best way to increase the survival is to keep does and fawns in the stable during the first two weeks to avoid confrontation with other species and cold weather. Perhaps the shelters described in Givskud Zoo might be implemented to provide both shelter from weather and protection from assaults. By using this approach the visitors can still experience the fawns and the fawns in turn will have to adapt to one habitat only.

A doe which shows obvious signs of stress should be removed before delivery or be placed in a pen for herself. Doing so in the summer, however, would remove most of the does from the public display, and hence affect the visitors' experience. Returning to winter breeding would remedy this, but then the indoor temperature, with special focus on the floor, must be secured to avoid hypothermia. This approach also will be complicated by the fact that most of the Savannah animals must also be kept indoor, which would lead to problems finding enough room for the pregnant blackbuck does.

5.7 Tips for future studies

Marking of the does, and preferably of the fawns as well, is very important. In this study the shaved patches on the back of the does were difficult to see in the black and white video sequences and wide angles, which in some cases made the logging of behaviours uncertain. Full and continuous video coverage of the entire pen would have been much preferred. This would require cameras with high resolution on one screen without the video splitter set on cycling, or parallel, synchronized recordings on different DVD recorders. Furthermore, I was forced to focus on the does because of the lack of markings on the fawns and the unforeseen frequency of allosuckling. In future studies I would recommend that the fawns are marked as well to be able to estimate the suckling duration for the individual fawns and not only duration on each doe. The normal routine, at Kolmården Zoo, with newborns is to make a hands-on check on day two or three. However, this normally is carried out while the females are let outside with the offspring left behind in the stable or while the offspring is lying out. In this case it was deemed not recommendable to enter the indoor pen for this check-out, and hence the fawns could not be marked. A way to solve this problem would be to have an adjacent box in which the does could be temporarily separated during this fawn check.

My intention was also to compare behaviours between Savannah exhibit and indoor pen which I was not able to do due to lack of data from the Savannah exhibit. Probably the best way to observe is to mount cameras in the Savannah exhibit as well, or in some other autonomic way log the behaviours or to organize a team of observers who could carry out continuous or at least much more frequent observations. In the early stage of planning, providing the doe with a radio collar was considered, but since no way of also marking the fawn could be found, this idea was abandoned.

6 Acknowledgements

I would like to thank my supervisor Mats Amundin. I would also like to thank Bengt Röken, Torsten Möller and the animal keepers for all their help with the animals, and Uno Wennergren and Nina Håkansson for their help with the data analysis. A special thanks to Thomas Jutebring at FLIR Systems for lending me a thermal camera, and also to Karolina Bördin for general support and building skills.

7 References

Altmann J (1974) Instantaneous sampling; Observational study of behavior: Sampling methods. Behaviour 49, 227-267.

von Borell E, Dobson H, Prunier A (2007) Stress, behaviour and reproductive performance in female cattle and pigs Hormones and Behavior 52,130–138.

Cabrera RJ, Rodriguez-Echandia EL, Jatuff ASG, Foscolo M (1999) Effects of prenatal exposure to a mild chronic variable stress on body weight, preweaning mortality and rat behavior. Brazilian Journal of Medical and Biological Research 32, 1229–37.

Cameron EZ, Stafford KJ, Linklater WL, Veltman CJ (1999) Suckling behaviour does not measure milk intake in horses, Equus caballus. Animal behaviour 57, 673-678.

Carolino N, Lopes S & Gama L (2004) Inbreeding and inbreeding depression in a churra Badana sheep flock. Archivos de Zootecnia 53(202), 229-232.

Cassinello J (2005) Inbreeding depression on reproductive performance and survival in captive gazelles of great conservation value. Biological Conservation 122, 453–464.

Chatfield C (1996) The analysis of time series: an introduction. 5th edition. Chapman & Hall, London.

Charpentier MJE, Widdig A & Albert SC (2007) Inbreeding depression in non-human primates: a historical review of methods used and empirical data. American journal of primatology 69(12), 1370-86.

Chaudhry AA & Tahir M (1988) Study of mother offspring behaviour of blackbuck (Antilope cervicapra) under captive conditions of Lahore Zoo. 8. Pakistan Congress of Zoology, 241-249.

Convention on International Trade in Endangered Species of Wild Fauna and Flora (2008) Appendices I, II and III
http://www.cites.org/eng/app/appendices.shtml (19 Feb. 2008)

Estes RD (1991) The behavioural guide to African mammals. University of California press, Berkeley.

Fonseca ESM, Massoco CO, Palermo-Neto J (2002) Effects of prenatal stress on stress-induced changes in behavior and macrophage activity of mice. Physiology & Behavior 77, 205– 215.

Garner JP (2005) Stereotypies and Other Abnormal Repetitive Behaviors: Potential Impact on Validity, Reliability, and Replicability of Scientific Outcomes. ILAR Journal 46 (2), 106-117.

Guo A, Nappi RE, Criscuolo M, Ficarra G, Amram A, Trentini GP, Petraglia F, Genazzani AR (1993) Effect of chronic intermittent stress on rat pregnancy and postnatal development. European Journal of Obstetrics and Gynecology and Reprodictive Biology(51), 41–45.

Homer CJ, James SA, Siegel E (1990) Work-Related Psychosocial Stress and Risk of Preterm, Low Birthweight Delivery. American Journal of Public Health(80), 173-177.

International Species Information System 2008
www.isis.org (2008-02-21)

Jiménez JA, Hughes KA, Alaks G, Graham L, Lacy RC (1994) An Experimental Study of Inbreeding Depression in a Natural Habitat. Science 266(5183), 271-273.

Kaiser S, Sachser N (2005) The effects of prenatal social stress on behaviour: mechanisms and function. Neuroscience and Biobehavioral Reviews 29, 283–294.

Laikre L, Ryman N (1991) Inbreeding Depression in a Captive Wolf (Canis lupus) Population. Conservation Biology 5(1),33-40.

Mason G, Clubb R, Latham N, Vickery S (2007) Why and how should we use environmental enrichment to tackle stereotypic behaviour? Applied Animal Behaviour Science 102, 163–188.
DOI: http://dx.doi.org/10.1016/j.applanim.2006.05.041

Mason GJ, Leipoldt A, de Jonge G (1995) Why do female mink with high stereotypy levels have slow-growing offspring? Proceedings of the

29th International Congress of the International Society for Applied Ethology. Exeter, UK: Exeter University, 133-134.

Merlot E, Couret D , Otten W (2008) Prenatal stress, fetal imprinting and immunity. Brain, Behavior and Immunity 22, 42–51.
DOI: http://dx.doi.org/10.1016/j.bbi.2007.05.007

Mungall EC (1978) The indian blackbuck antelope: A Texas view. College station, Texas.

Mungall EC (1991) Establishment of lying out: an example for blackbuck (Antilope cervicapra). Applied Animal Behaviour Science 29, 15-37.

Patin V ,Lordia B, Vincenta A, Thoumas JL, Vaudryb H, Castona J (2002) Effects of prenatal stress on maternal behavior in the rat. Developmental Brain Research 139, 1–8.

Ranjitsinh MK (1989) The Indian blackbuck. 1st edition. Natraj publishers, Dehradun.

Roulin A & Heeb P (1999) The immunological function of allosuckling. Ecology Letters 2, 319-324.

Sørensen G, Randrup A (1986) Possible protective value of severe psychopathology against lethal effects of an unfavourable milieu. Stress Medicine 2, 103-105.

Wainwright PE (1981) Maternal Performance of Inbred and Hybrid Laboratory Mice (Mus musculus). Journal of Comparative Physiology and Psychology 95(5), 694-707.

Appendix I

The male has long spiralled twisted horns and stands 74,6 cm at the shoulder (mean value male) while females in most cases are hornless and stands about 70,2 cm (mean value female) at the shoulder. In rare cases in northern India male blackbucks can grow as tall as 84 cm. At times horned females have been observed. Mostly those females are in an unbalanced hormonal state and sterile. Males may also develop deformed horns if castrated or otherwise hormonal unbalanced and might be mistaken for horned females.

An adult male weighs 35-43 kg while females weigh about 23-32 kg. A newborn fawn weighs 3-4 kg.

The male fur is brown on the back and head where it darkens with age to near black. Females are orange-tan while fawns are brown at birth, passing a lighter cream-coloured stage before developing the orange-tan at adolescence.

A blackbuck may live for 15-16 years but mortality increase drastically at 6-7 years of age (Mungall 1978).

The habitat preferred is short grass tracts whit grass no higher than 40 cm. Proximity to shrubs and bushes is avoided as well.

The Indian blackbuck is a tropical animal and may come into estrus at any time of the year. However, in India, a peak was seen just after the monsoon had provided food for the mother and cover for the fawn.
In Indian areas where blackbuck has been studied (e.g. Velavadar National Park) temperatures ranging from 19,3 ^{o}C to 32,8 ^{o}C has been reported depending on season. In Kanha National Park the temperature never exceeds 32,8 ^{o}C and may go down to -2,5 ^{o}C (Ranjitsinh 1989). Blackbucks have been also introduced in Texas, US, where temperatures at some points may be as low as -18 ^{o}C. It is not uncommon to observe blackbucks with club formed ears due to frost-bite. (Mungall 1978).

Both male and female blackbuck has been seen in mixed groups, single sex groups, alone or in pseudo-harems. Mixed groups and pseudo-harems may be hard to separate when bucks older than adolescents join in unless one knows the identity of the territorial bucks. Pseudo-harems may be misinterpreted as harems although bucks do not keep harems. Instead groups pass through buck territory each day and meanwhile the territorial buck join the group and court females.

It is doubtful that a single animal is the leader of a group. It is most likely that the animals know each other individually but that none is in charge. The activity is determined by “voting”. If one animal starts in one direction and others follow the rest of the group will most likely follow.

Appendix II

Lying out position 1. Between the roots in a stump.

Lying out position 2. A shallow cavity near the outer fence

Lying out position 3. Between a stump and a large stone in a ~45^{o} slope.
Lying out position 4. Between two medium large stones in a slope.

Lying out position 5. Behind a very large stone near the outer fence.

Lying out position 6. In a crack of a large stone in the centre of the exhibit.

Lying out position 7. In a stony slope near the stable gates.
Lying out position 8. Next to a flat rock.