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Predation on cattle in Kenya and its effect on the stress level of the animals in the exposed herd

Sveriges lantbruksuniversitet
Fakulteten för veterinärmedicin och husdjursvetenskap

Predation on cattle in Kenya and its effect on the stress level of the animals in the exposed herd

Rovdjursattackers påverkan på stressnivån hos utsatta kor i Kenya

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Omfattning: 45 hp

Kurstitel: Master thesis in animal science

Kurskod: EX0719

Program: Animal Science - masterprogram

Nivå: Avancerad E45

Utgivningsort: SLU Uppsala

Utgivningsår: 2017

Omslagsbild: (Linda Eriksson)

On-line publicering: http://epsilon.slu.se

Serie: Studentarbete/Sveriges lantbruksuniversitet, Institutionen för husdjurens miljö och hälsa, nr.716, ISSN 1652-280X

Nyckelord: Predation, kortisol, håranalys, kronisk stress, stress, Kenya

Key words: Stress, predation, cattle, hair analysis, Kenya, cortisol

ABSTRACT

Predation on livestock affects the human-carnivore conflict and can also lead to great economical loss for farmers. Lions are the predator that attacks the most cattle in Kenya, but there are also attacks from hyenas, leopards and jackals. Predation on livestock is a worldwide problem and it is therefore of big importance to investigate how the predator attacks are affecting the cattle that survived in the exposed herd. If predator attacks leads to chronic stress in the cattle, it can affect the reproduction cycle of the animals, the health and also their productivity. In this study two herds that had been attacked by predators during the last 10 months (one herd with cows and one herd with steers), and also two control herd that had not suffer from any attacks during the same time were chosen to be analyzed for hair cortisol as an indicator of stress. Hair samples were collected from the tail switch from 10 random individuals from each of the four herds and then analyzed for cortisol, as cortisol gets deposit in the hair constantly as it grows. The result in cortisol indicates that there was no significant difference between attacked and non-attacked herds or between steers and cows. There is also no significant difference in cortisol levels between animals with different body condition scores. There was a significant difference in cortisol levels in the color of the hair, with light hair containing the highest amount of cortisol.

SAMMANFATTNING

Rovdjursattacker på produktionsdjur påverkar konflikten mellan människor och rovdjur samtidigt som det kan leda till ekonomiska förluster för djurägaren. Lejon är det rovdjur som attackerar flest kor i Kenya, men det sker även attacker av hyenor, leoparder och sjakaler. Predation på produktionsdjur som vistas utomhus är ett stort problem världen över och det är därför av stor vikt att utreda hur mycket en rovdjursattack påverkar flocken långsiktigt. Om produktionsdjuren blir kroniskt stressade efter attacken kan det påverka produktionen, hälsan och reproduktionen av djuren i flocken. I denna studie valdes två flockar med stutar ut, en flock som hade blivit rovdjursattackerad dem senaste 10 månaderna, samt en kontrollgrupp som inte hade blivit utsatta för någon attack under samma tidsperiod. Även två grupper med kor, en som blivit attackerad och en som inte hade blivit attackerad valdes ut. Alla djur i denna studie befann sig i Ol Pejeta Conservancy i Kenyas Laikipia distrikt. Hårprover togs från svanstippen på 10 slumpmässigt utvalda individer ur vardera flock och analyserades för kortisol, då kortisol lagras i hårstråna när dem växer. Resultatet tyder på att det inte finns någon signifikant skillnad i kortisolhalt mellan attackerade flockar och icke-attackerade flockar, inte heller mellan stutar och kor. Det finns heller ingen signifikant skillnad mellan djur med olika mycket hull. Det fanns en signifikant skillnad på kortisol nivåer i olika färger på håret, där ljust hår innehöll större mängd kortisol.

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INTRODUCTION

Predators on livestock

Livestock loss due to predation is a global problem (Amador-Alcalá *et al.* 2013, Aryal *et al.* 2014, Frank *et al.* 2016) that affects the human-carnivore conflict (Thorn *et al*. 2012). There are different conditions and approaches to reduce the risk of predation on livestock over the world. When the livestock live integrated with the wildlife, the use of guarding dogs (*Canis*) is one way to reduce attacks, both during night- and daytime (Ogada *et al*. 2008). When it comes to predator attacks on cattle (*Bos indicus*) in Kenya, the most common predator is lions (*Panthera leo*). Leopards (*Panthera pardus*) and hyenas (*Crocuta crocuta*) are also big threats. 75% of the attacks from lions, leopards and hyenas occur during nighttime when the cattle are in their night time corrals called bomas. The remaining 25% of the attacks occur during daytime when the cattle are out grazing (Kolowski & Holekamp, 2006). Lions mostly attack adult cattle, while leopards attack calves (Ogada *et al.* 2003).

Patterson *et al*. 2004 performed a study on predation on cattle in Kenya and observed that an average of 1.36 cattle was killed per attack performed by lions. 1.07 was the averaged cattle loss at attacks from hyenas. The cattle loss due to predation can have great negative effects on the economy of the cattle-owner, which further can affect the human-carnivore conflict.

One effective way to reduce the risk of predation on livestock during nighttime is to keep the animals inside good bomas. Ogada *et al.* 2003 observed that some lions jumped in to the boma and killed their pray, while others took their pray after the cattle got scared and broke out of the boma. Therefore a good boma is constructed so that it keeps the panicking cattle inside at the same time as it prevents the predator the get in. Bomas can be made out of solid wood or stone, wire fences or out of thornbush. Bomas made out of thornbush is often built with different "rooms" and it is effective in keeping scared cattle from breaking out, because of the distribution of the pressure (Ogada *et al.* 2003).

Other factors that can affect the frequency of the attacks during night are the amount of animals in the bomas and how far it is to the next boma (Kolowski & Holekamp, 2006). Herders also reduces the risk of cattle loss due to predation by ensuring that there will be no stray cattle during the daytime and the number of people around the boma is strongly correlated to the attack rate from lions and leopards, showing that the more people there is around the boma, the less predation attacks occur (Ogada *et al*. 2003).

Stress

When an organism is exposed to a stressor, the organisms homeostasis is threatened and the innate response of the organism will help to regain homeostasis. The innate response take place in the central nervous system (CNS) and will make the organism alert and focused by facilitate the neural pathways to adoptive functions, while it will inhibit pathways to nonadoptive functions such as eating and growing (reviewed in Chrousos, 2009).

There are two different types of categories of stress, acute stress and chronic stress. The acute stress response activates when the animal is exposed to a stress-stimuli for a short time. The acute stress response contains both the fight-or-flight response, and a glucocorticoid response. The fight-or-flight response contributes to an increase in heart rate and blood pressure and is followed by the slower glucocorticoid response (Dickens *et al*. 2010). One of the hormonal response systems that are important when an animal is exposed to a stress-stimulus is called hypothalamic-pituitary-adrenocortical axis (HPA). When neurons in the paraventricular nucleus of the hypothalamus exude corticotrophin-releasing hormones (CRH), the HPA system gets activated. When this molecule gets to the anterior pituitary gland, the response will be the release of adrenocorticotropic hormone (ACTH). When this hormone gets to the adrenal glands, cortisol will be released (Miller *et al.* 2007). The cortisol release decreases after the stress-stimuli vanished by negative feedback. These responses are supposed to help the animal survive, and unnecessary system gets turned off during the time that the acute stress response is activated.

Chronic stress can occur when there is an eliciting stimulus that remains in the environment for a longer time, or when an individual experiencing something bad for a short moment, but the experience leaves a feeling of threatening for a long time after (Miller *et al.* 2007). If an animal is chronically stressed, the acute stress responses itself start causing problems for the individual. If the fight-or-flight response is activated for a long time, the animal has a higher risk for myocardial infarctions and hypertension. If the animal has high levels of cortisol during a long time, the reproductive hormone axis can get affected, and also the immune system (Dickens *et al.* 2010).

There are different methods to evaluate stress in an individual. Cortisol can be measured in blood, urine, faeces and saliva samples. Blood and saliva samples have a short time-frame of minutes, while urine and faeces can give a longer time-frame up to a day. The collection of the samples itself can be stressful to the animal and affect the result depending on the method. Blood samples has a high risk to be stressful to the animal, and the time-frame is just minutes, resulting in that the value of cortisol is high in the samples because of the stress that the needle is producing. When collecting samples to analyze for cortisol using any of the methods named earlier, circadian rhythm in cortisol should be considered. The amount of cortisol is peaking after light onset and offset (Novak *et al*. 2013).

Another way to investigate chronic stress in an individual is to measure the cortisol concentration in the hair (Gow *et al.* 2009), because cortisol is constantly deposited in the hair as it grows. This method can give knowledges in the HPA activity over months (Novak et al. 2013) instead of 12 hours as fecal samples for cortisol will show in ruminants (Möstl & Palme, 2002). To evaluate if an individual is suffering from chronic stress with help of hair samples is a quite new technique. In humans it is observed that factors as sex and adiposity can affect the results. Therefore those factors have to be considered when hair samples are taken (Wosu *et al.* 2013).

The amount of cortisol differs in different colors of the hair on the cattle, with white hair containing the highest amount of cortisol. In a study on Holstein cattle it has been observed that the tail switch was the best location for hair sampling. This because the hair growth was fast, the color of the hair was white and it was easy to access (Burnett *et al.* 2014). Mayer and Novak (2012) suggest that the sample should be collected from a place where self-grooming is hard, so the hair is as uncontaminated as possible. The collection of hair should be made by a razor or by scissors, because follicles can follow and the blood can contaminate the sample if the hair is pulled from the animal (Mayer & Novak, 2012).

Aim of the study

Predators attacking livestock is a worldwide problem and because of the fact that stress can affect the productivity of the animal, there is important to know how much predator attacks on a herd affect the animals.

Therefore the object of this study is to investigate if cattle herds that been attacked by predators during the last 10 months suffer from higher stress hormone levels than herds that have not suffer from any attacks during the same time.

The hypothesis of this study is that attacked animals should have a higher level of cortisol in their hair, as an indicator that these animals are more stressed than the animals that have not suffer from any predator attacks during 2016.

Another hypothesis is that the steers should be more stressed and therefore has a higher level of cortisol in their hair than cows.

The third hypothesis is that animals with low body condition score should have higher levels of cortisol in their hair that animals with high body condition score.

MATERIAL AND METHODS

Place of the study

The data needed for the study was collected in Ol Pejeta Conservancy, which is located in Kenya's Laikipia District. The conservancy covers a 365 km^2 land area and the collecting of data was carried out between the 14^{th} and the 28^{th} of November 2016. 53% of those 365 km^2 land that Ol Pejeta Conservancy covers is open bushland, 27% dense bush, 22% grassland and the containing percent is marsh or rivers (Ol Pejeta Conservancy, 2016).

Inside Ol Pejeta Conservancy they have a breeding program for one breed of *Bos indicus* - the Boran cattle (Ol Pejeta Conservancy, 2016). Ol Pejeta also buys cattle from outside-farmers. These cattle are not purebred Borans, but they are all *Bos indicus,* and are therefore called “Zebu” when they are taken in to Ol Pejeta Conservancy. *Bos indicus* evolved in India from *Bos namadicus* and are now used in Africa, Australia and South America (Philips, 2010).

Data collection

Interviews

The head of the livestock department in Ol Pejeta Conservancy supported this study with the documentation of the cattle lost inside Ol Pejeta Conservancy from the past one and a half years (from 6th of June 2015 to 14th of November 2016). Those documents included date when the attack appeared, the category of cattle that been lost (calf, steer, bull, heifer or cow), the dam's ID number, the cause of death, the herder, the location and the person reporting the injury or death. In most cases the predator was documented, otherwise it was categorized as "unknown predator", and in some cases it was documented if the attack occurred during the day or the night.

Ol Pejeta Conservancy employs approximately 100 herders to take care of the 6000 cattle that live inside the conservancy. The herders know the terrain, and walk the cattle to the water points and pastures, and then back to the bomas at night. (Ol Pejeta Conservancy, 2016). The documents of the cattle loss during the last one and a half year were analysed and then 22 of the herders in Ol Pejeta Conservancy were interviewed from a questionnaire about the predator attacks they experienced during 2016.

Ol Pejeta Conservancy has three different types of so called bomas to reduce the loss of cattle to predators. The bomas are either mobile wire fences made out of steel (as seen in picture 1), electric bomas or light bomas. The electric bomas are made out of one electrical wire that surrounds the cattle herd. The light boma is no actual enclosure, but have strong spotlights that keep the predators away.

Hair sampling

From the results of the summarization of the cattle loss documentation and the interviews of the herders, four herds were chosen for cortisol analysis, see table 1. Two herds were chosen as attacked groups since, - both hade documentations on the attacks and confirmation from the herders. The two herds that were chosen as control groups had no recordings of attacks during the previous year.

Table 1: Herds chosen to be analyzed for cortisol amount in the hair. The cows were attacked in June 2016, and the herd of steers were attacked in November 2016 according to the documentation and one attack occurred (according to two herders, but not mentioned in the documentation) in June-July 2016.

Herd	Category of animal	Amount of animals in herd	Nr. Of attacks during 2016	Attack occurred inside or outside boma
Zebu attacked (ZA)	Steers	211-214	2	Outside the boma, during daytime
Zebu control (ZC)	Steers	212	0	
Boran attacked (BA)	Cows with calfs	213	1	Outside the boma during daytime

Boran control (BC)	Cows with calfs	112	0	

The tail switch was cut with a scissor from 10 randomly chosen animals in each of these four herds. The body condition of the cattle was documented from the scale in table 2.

When hair samples were taken, the cattle were passed through a fenced passage that the cattle were used to walk through, once every week when they got sprayed with tick agent.

Table 2. Categorization of the condition of the animal. (Adapted from Edmondson *et al*. 1989)

Score 1	Ribs, backbone, hips and shoulder bones are clearly visible.	
Score 2	Ribs, backbone, hips and shoulder bones are visible.	
Score 3	Hips are faintly visible, and ribs are not visible at all.	
Score 4	Hips, Shoulder, ribs are not visible.	
Score 5	Hips have fat deposit and ribs not visible at all.	

The hair was then washed with water and dried. Then the samples were washed once more with alcohol and then transported to Sweden. All necessary permits to import the hair samples were obtained and granted before sending the samples to Sweden.

80 mg of hair was taken from each individual hair sample and was put into 15 ml conical tubes. Approximately 1 cm of the hair closest to the skin of the animal was used. 5 ml of isopropanol was added in each tube, and then each sample was vortexed for 3 minutes. This step was repeated 2 times for each sample. Then the samples were dried in room

temperature for approximately 36 hours. Because Burnett *et al.* 2014 observed a difference in the amount of the cortisol in different colors of hair, the hair color was documented for each sample as seen in table 3.

Table 3. Categorization of haircolor.

Dark	All hair was in the same color and the hair was either black or brown.
Mixed	The hair in the sample had both light and dark color.
Light	All hair was in the same color and the hair was either white or light yellow.

50mg from the cleaned and dried hair samples were then putted into 2 ml tubes together with 3 chrome steel balls (3.2mm-diameter). All samples were then frozen in liquid nitrogen for 2 minutes each, and then a bead beater was used to pulverize the hair. The samples were in the bead beater for a total time of 3 minutes.

For steroid extractions, 1.2 ml of methanol was added to each of the samples, and then they were put in a cradle for 21 hours in room temperature. The samples were then centrifuged at 7000 x g for 2 minutes, then 0.8 ml of the supernatants were transferred into Eppendorf tubes (1.5ml). The samples were centrifuged once more at 10 000 x g for 5 minutes and then 0.6 ml of supernatants were transferred to new 1.5ml Eppendorf tubes. For the next 29 hours, the samples were placed on a heat block at 38*C. 0.2 ml of phosphate-buffered saline (BPS) was added to each tube and then all samples were vortexed. Sample were then stored at -20 degrees until cortisol analysis.

Before the cortisol analyses, samples were taken out and defrozen in room temperature. Then an Expanded Range, High Sensitivity Salivary Cortisol Enzyme Immunoassay Kit from Salimetrics * (item No 1-3002) was used to determine the concentration of cortisol in the samples.

This kit is designed to measure cortisol in saliva, and therefore the result received is in ug/dl liquid. The result has then to be converted into pg per mg hair instead, done by using the formula $(A/B) * (C/D) * E * 10\,000 = F$. A is the result from the test in ug/dl, B is the weight of the hair in mg, C is the volume of the methanol (in this case 1.2 for all samples). D is the volume of methanol that was dried together with the hair (0.6 for all samples), and E is the volume of BPS used (0.2 for all samples). Finally, F is the result of cortisol concentration in pg/mg (Mayer *et al*. 2014).

All samples were analyzed in duplicates and every 10th sample was analyzed as a double extraction analysis to assure that the method was working.

Statistical Analyses

All analyses were made in MiniTab, 2017 version. Because of the small sample size in each herd, the body condition score was divided in to only two categories, those with body condition score 2.0-2,5 as one group, and those with 3.0-4.0 as the other group.

The mean of the two results in cortisol level from the double extraction samples were used in the analysis.

The cortisol level in the hair was summarized by mean ± standard error (SE). ANOVA (Analysis of Variance) was used to calculate if cortisol levels in hair differed in relation to predation on the herd or not, the breed and gender of the herd and the color of the hair in the sample, this was done with a 95% confident interval (CI). Factors used were attacked/non attacked, steer/cow, hair color and body condition score. Also, attacked/non attacked was tested with in each group of cattle, i.e. steers and cows.

All hair samples per head of cattle were pooled to one value before used in the statistical analysis.

RESULTS

Table 4. Number of cattle loss and type of predator according to the documentations records of 2015-2016.

	Lions	**Hyenas**	**Leopard**	**Jackal**	**Unknown predator**	**Cattle loss to predation**
6/06/2015 – 31/12/2015	46	6	10	3	6	71
1/01/2016 – 14/11/2016	32	4	4	4	21	65
Total	78	10	14	7	27	136

During 2016, 80 cattle were lost due to predation in Ol Pejeta Conservancy. 50% of the attacks were performed by lions and the rest of the attacks by hyenas, jackals, leopards and unknown predator.

The answers from the interviews with 22 herders, is seen in table 5. Only 12 out of the 22 herders claimed to have experienced an attack during 2016. Some of them claimed to have had multiple attacks.

Table 5. The answers from the interviews of herders.

	Total	Lion	Leopard	Hyena	Jackal	Unknown
Number of attacks	19	17	1	2 (lions as well)	1	0

In the documentations received from the head of the livestock department in Ol Pejeta Conservancy, the type of boma was not documented. In 89 out of 136 cases, the time of the attack was recorded, as shown in figure 1. Two categories were used; the attack was documented to occur during night or during day. Night time means that the cattle were inside the boma and day time out of the boma.

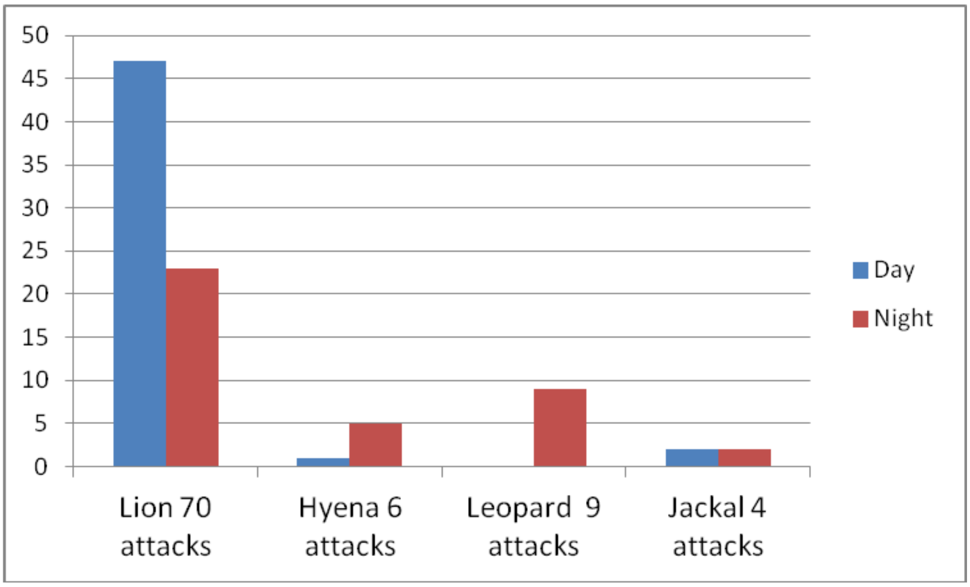

Figure 1. Time of the attack from the documentation of cattle loss

Ol Pejeta Conservancy has one area called Sirrima, which is supposed to be free of predators. The cattle in this area are loose at night, and not put inside a boma. Therefore, the herders in Sirrima was not interviewed, and the herds where not considered for cortisol analysis in hair. According to the documentation records over the past one and a half year, some cattle have been lost to predators in this area although it should be predator free. During the last six months of 2015 the loss of cattle to predation in this area was 13, and between January and the 14th of November 2016 it was also 12 cattle lost. Between the 6th of June 2015 and the 14th of November 2016, that is a total loss of 25 cattle. That was 18% of the total cattle loss due to predation.

Table 6. Result of cortisol analysis in hair when observing all four groups.

Index	Cortisol concentration (pg/mg)
Range	2.827 – 16.949
Median	4.874
Mean ± SE	5.744 ± 0.412

The attacked group had a cortisol mean of 5.547 ± 1.927 pg/mg hair, and the herd who had not suffer from any attacks during 2016 had a mean of 5.941 ± 3.187. The mean and SE for each of the four groups will be displayed in table 7.

Table 7. The mean and SE of the four different groups of animals (BA – Boran attacked, BC – Boran control, ZA – Zebu attacked and ZC – Zebu control)

Group	Mean ± SE (pg/mg)
BA	6.193 ± 0.521
BC	4.948 ± 0.591
ZA	4,901 ± 0.648
ZC	6.930 ± 1.260

There was no significant difference in cortisol levels in the hair when analysis all attacked animals against all control animals (p-value = 0.7). There was only a significant difference in the color of the hair (p-value = 0.000) with light hair containing a higher amount of cortisol. There is no significant difference in body condition score (p-value = 0.189) or in the breed/gender (p-value = 0.114). The body condition score was only documented on the zebu steers. All steers got a body condition score between 2 - 4.

When comparing ZA against ZC, there was a statistical tendency between attacked and non-attacked Boran cows (p = 0.057). For Zebu steers, there was no such affect between attacked and non-attacked animals (p = 0.435), as seen in table 8.

Table 8. P-value when comparing BC (Boran control) and BA (Boran attacked), and the p-value when comparing ZC (Zebu control) and ZA (Zabu attacked).

Groups	P-value
BA vs BC	0.057
ZA vs ZC	0.435

DISCUSSION

57% of the cattle loss due to predation according to the documentation records (from the 6th of June 2015 to 14th of November 2016) was due to lions. Hyenas caused 7% of the total cattle loss, leopards 10% and jackals 5%. The resuming 21% of the cattle was lost to unknown predator. As Kolowski & Holekamp (2006) observed, lions are the predator that causes the highest amount of cattle loss in Kenya.

The goal with the interviews of the herders was to get an understanding of what kind of vegetation it was when the herd got attacked (high or low grass, bushy or open fields) and if it was one or more predators that attacked. These factors were not documented in the records but could be of importance when deciding which herds to use and to understand the patterns of the attacks. The interviews with herders gave different results than the documented records given by the head of the livestock department. Some herders claimed that their herd did not suffer from any predator attacks during the year of 2016, even though it was documented that it had. The interviews were therefore limited to just deciding which herds to use in the cortisol analysis in the way that the herder interview and the documentation corresponded. The herds that were chosen in the cortisol analysis for this study had both attacks recorded in the given documentations of cattle losses and the herder described an attack or attacks when being interviewed as well.

The sample size in this study was small overall (N=40) and then it was also divided into four groups, so each group had an N=10. It would have given a more reliable result if the sample size was bigger and it would also have given a more reliable result if the two attacked herds and the two control herds were of the same gender and breed (preferably Boran). If all four herds should have been Boran cattle, the gender bias, and the breed and history (growing up in Ol Pejeta Conservancy with high amount of predators or not) bias could have been eliminated. Because of the short time-frame of this study and the fact that hair samples only could be collected when the herds were scheduled to get sprayed with tick agent, made it hard to get all those variables to correspond.

The mean ± SE (see table 6) of cortisol in the hair of all cattle (5,744 ± 2,607 pg/mg hair) in this study is a bit lower than the amount that Burnett et al. (2015), Burnett *et al.* (2014) and Gozález-de-la-Vara *et al.* (2011) observed in dairy cows (9.8 ± 3.7 pg/mg hair, 5.7 ± 1.7 pg/mg hair and 12.5 ± 1.85 pg/mg hair respectively), but higher than Comin *et al.* (2013) observed (mean of 3.29 pg/mg). It is close to the cortisol level that Moya *et al*. (2013) found in beef cattle, ranging from 0.30 to 5.31pg/mg. The amount of cortisol in this study therefore seems like a reliable result. Compared to the amount of cortisol found in other cattle when doing analyses of the hair, the cattle inside Ol Pejeta Concervancy do not seem to have a higher cortisol level than other cattle living inside barns.

There was no significant difference in cortisol levels between the herds that have been attacked during the last year and the herds that have not suffer from any predator attacks during 2016, if all herds are compared to each other. There was a statistical tendency in the amount of cortisol when the BA and the BC were compared with each other, but not when ZA and ZC where compared to each other. The attacked cow herd was exposed to the attack in June 2016 and the steers were attacked in November (according to both herders and the documentation of cattle loss) and sometime in June-July according to two herders when interviewed individually without chance to hear each other answer. Attacks were performed by lions in both cases. Only one centimeter of the hair from the cut side, closes to the skin, was used for cortisol analysis and one possibility is that the cortisol levels had been high in the hair of the attacked herds when the attacked occurred and some time after that, but that it had reduced to normal levels again before the growth of the last centimeter of hair, closest to the skin. The attack in November could on the other hand be too close to the collection of hair samples (14 days between the attack and the collection of hair samples). Scissors were used to collect the hair, and therefore it was hard to cut the hair directly against the skin, and therefore the hair that had grown since the beginning of November were probably too short to reach with scissors. To get closer to the skin without risking to cut the skin, a razor could be used.

There was also no significant difference between steers and cows, which was suspected because of the fact that the steers was raised outside Ol Pejeta Conservancy, and therefore not used to the same amount of predators in the surroundings. The cows and steers were also of different age. Other studies have found that age affected the cortisol-level in the hair, with younger animals having a higher amount of cortisol (Gozález-de-la-Vara *et al.* 2011). Also, the gender itself was suspected to affect the result, with earlier findings in humans (O'Brian *et al*. 2012) that females have a lower amount of cortisol in the hair than males. One possible explanation to the non-significant difference between attacked herds and non-attacked herds is that all animals are used to the high density of predators and the smell and sound of them. According to the documentations of cattle loss, the herd with most frequent attacks, had four attacks during 2016. If the cattle are hearing predators daily, but only gets attacked a few times a year, it might not lead to chronic stress.

An explanation to the result that there is no significant difference between steers and cows, could be the method of the study. Those two groups have too many differences between each other (gender, breeds, growing up with different predator density in the surroundings). Even though all these factors are pointing on steers to be more stressed, maybe the breed of the steers has harder to get stressed than Boran. Even though the presumed Zebu individuals can be Boran mixed breeds, there can be a big difference in cortisol levels in hair, as Peric *et al*. (2013) observed in different crossbreed generations. This has to be further investigated to draw any conclusions.

As observed in earlier studies (Gozález-de-la-Vara *et al*. 2011, Burnett *et al*. 2014) there was a significant difference in the cortisol level depending on the color of the hair sample ($p \leq 0.001$). The highest amount of cortisol in the present study was as in earlier studies found in the lightest hair.

To get a good understanding of how predator attacks really affect the cattle, behavioural studies should have been added as well. To observe if the attacked herds are for example performing more of vigilance behavior or lying down less than the herd that have not suffer from an attack can give a better understanding of how the predator attacks affect the individuals that survives.

How to improve the study

The herders were interviewed for all attacks that happened to their herd during a year back. There were some difference in the data from the livestock department in Ol Pejeta Conservancy and the interviews with the herders, and it could possibly be reduced by doing the interview with fixed question directly after an attack occurred.

The cattle were not used to close human contact from strangers, and cutting the hair caused a lot of stressful behavior in some herds. When the samples of hair were taken, some cattle tried to run away, pushing the cattle in front of it and/or kick. It was not possible to get some individuals to stand still during the haircutting, which resulted in different amounts of hair samples from different cattle. When preparing the samples for cortisol analysis, the same amount and length of hair was used from each individual.

Otherwise it would affect the time scale of the samples, because when the samples are made, they are based on weight. Meaning that the samples from cattle where we did not manage to get so much hair, should give a longer timescale if we used longer of each hair. The density of the hair can differ between both individuals and breeds (Peters & Slen, 1964). This could have an effect on our results, because our samples were not cut from the exact same sized area from each cattle, but we still used the same amount of hair in our samples. The age of the animal also can affect the density of the hair (Peters & Slen, 1964). We had the age factor in mind when we selected herds to cut hair from and choose two as similar herds as possible to compare with each other.

In this study the hair samples were taken when the cattle were passing through the fenced passage they walk through when they were getting sprayed with tick agent. They were therefore used to the passage. The cattle tried to turn around in the passage, stopped in it and some of the cattle tried to go through the fence. Therefore there are reasons to think that the cattle did not appreciate to walk through the passage, and therefore it could have made the sampling collection harder than it had to be. If the animals should have been calmer, the size of the area cut could have been better controlled, and the distance from the skin could have been more accurate.

The cortisol amount can differ in different colors of the hair (Burnett *et al.* 2014) and different location of the body (Mayer & Novak, 2012, Burnett *et al.* 2014). We only collected hair from the tail switch and in the same location of the tail switch as far as possible. Because of the stressed animals it was not possible to take the samples from the exact same location on each animal. The cattle in Ol Pejeta Conservancy also differed a lot in color and the samples are from different hair colors. If a similar study would be done again, the hair should preferably be taken from individuals that have been in close contact with a predator and survived. Then have control animals with the same color of the tail, which lives in a herd that have not been suffering from any attack in the last year. That could give a more proportional distribution among colors among the samples.

In the result of the amount of cortisol in the hair in this study, each 10^{th} sample was a double extraction, and the result in those duplicate differed. One possible explanation to that could be that one out of the two samples were dirtier than the other, but in this study the hair was washed more than recommended in other studies, because of the transportation from Kenya to Sweden. Another possible explanation could be that the hair sample from the cattle had different colors on the hair, and one extraction had more light hair that the other one, and therefore the result differ within the hair sample.

The hair samples in this study were collected from the tail switch. According to Mayer and Novak (2012), the hair samples should be taken from a place where self-grooming is hard, to reduce the contamination risk. If this should have been considered, the best place to take the samples should be on the forehead of the cattle as Comin *et al*. (2013) did. The hair of the breed of cattle on this study was very short in all areas except for the tail switch, and therefore we decided to take the hair samples from there.

Because of the time limit in this study, the cattle only got cut for tail hair once. This can have a negative effect on the result because of the fact that it is hard to tell how fast the hair grows. It is therefore hard to say what time period that the sample result is showing. It would give a better result to shave the animal first, and then cut the hair after it has grown out again (Mayer & Novak, 2012). Then the time period for the result would be easy to tell. According to the documentations received from the livestock department, there was not common that cattle survived after physical contact with a predator. If the herders manage to scare the predator away, the damage on the animal is often so big that the animal needs to be euthanized. It is hard to know in which herd the next attack will occur, and therefore all cattle inside the conservancy need to be shaved if it should be possible to know that the cattle have been attacked during the hair growth. Another option would be to shave all cattle in a specific herd and wait until an attack appears in that specifically herd.

Choice of method for measuring stress

There are different methods for measuring stress. IgA, alpha-amylase and free cortisol are different components that can be measured to get an understanding it the animals stress level. All these component can be measured in salivary samples of an individual (Paszynska *et al.* 2016). Taking salivary samples gives a measurement of the stress level at the moment when the sample was taken, and because of the behaviors of the animals when they went through the passage where samples were collected in this study, indicated that a salivary sample would give an unreliable result. Cortisol can be measured in other ways than in salivary samples. Saliva, blood, faeces or urine sample could be taken instead of hair (Beerda *et al.* 1996, Novak *et al*. 2013), but those samples represent the cortisol level for some minutes or hours. To see if an animal is chronically stressed; multiple samples have to be taken over time (Mayer *et al*. 2014). This would be both expensive, time consuming and stressful for the animals. The cattle was not used to strange-human contact and acted stressed when the hair samples were taken. The stress there and then did not affect the cortisol in the hair samples, but it could affect the result in the other methods if those were to be used. Hair samples could also been stored and transported easier that blood, saliva, urine or faeces. Cortisol in hair is stable and can be stored up to 11 months in room temperature without affecting the cortisol-level (González-de-la-Vara *et al*. 2011). Urine samples would also claim to keep the test animal individually and to get a reliable result; all the urine during a 24 hour period should be collected. Because of the circadian rhythm of cortisol during the day (Novak *et al*. 2013) blood, urine and saliva samples has to be taken multiple times during the same day and that was not possible in this study.

The use of cortisol analyzes from hair is a reasonably new method to evaluate chronic stress in individuals. Studies have been performed on cat and dogs, where the amount of cortisol in the hair has been compared with both saliva and faeces samples that indicate a positive correlation in cortisol levels (Accorsi *et al.* 2008).

Trevisan *et al*. 2017 observed a difference in cortisol amount depending on the body condition of pigs, showing that lane pigs had a higher value of cortisol in their hair. Because of the missing value of body conditioning score of 12 of the Boran cattle in this study, the body condition score was not calculated for in the analysis of the cows. In the Zebu steers the body condition score was calculated for in the analysis, but without significant results ($p = 0.189$). The body condition score among the Zebu steers did not differ dramatically, with all animals having a score between 2 and 4, with a mean of 2.8.

If greater sample size where used, the body condition score might have given a significant difference.

Predator prevention and further studies

Inside Ol Pejeta, the most common boma is the one made out of steel fences. This is because of the fact that they are easy to move to where the grass is growing. A traditional thornbush boma is in contrast always in the same place, even if the boma is effective in keeping scared cattle inside it is unpractical when it comes to mobility (Ogada *et al.* 2003). The animals are standing as tight as possible in the steel fenced boma, preventing gaps for predators to jump in and preventing the cattle to move too much and tear down the boma if they are scared by a predator outside the boma (Richard van Aard, personal communication). Steel bomas can be regulated in size after the herd, so that the boma never has much space for the predators to jump in. Bomas out of thornbush is hard to regulate in size after the herd. To investigate if the cattle have different amount of cortisol in the hair depending on the different types of bomas could be an idea for further studies. Most of the attacks from lions inside Ol Pejeta Conservancy occur during daytime (see table 6) and one explanation to this could be that it is hard to attack the cattle during nighttime because of the construction of the boma.

In the predator free area in Ol Pejeta Conservancy, called Sirrima, there was a total loss of 25 cattle between 06/2015 and 11/2016. The cattle in this area are free during the night, and not put into bomas. The density of predators in this area should be lower than the rest of the Conservancy, and therefore it is an indicator that the mobile fences bomas that the cattle spend the night inside is having a positive effect in protecting the cattle from predators. Kolowski & Holekamp (2006) observed that 75% of the attacks on cattle occurred during night time but the good constructions of the bomas mostly used in Ol Pejeta Conservancy could be a possible explanation to why the majority of attacks from lions (68% of the attacks that have documented time – 70 attacks) in Ol Pejeta Conservancy appears during the day when the cattle is out gracing.

Another idea for further studies could be to investigate how the herd sizes affect the amount of cortisol in the hair. It has been observed earlier that animals decrease their vigilance behavior as the herd size increases (Rushen *et al*. 2008). The sizes of the herds used in this study was almost the same for three of the herds (ZA - 212, ZC - 212 and BA - 213) but the forth (BC - 112) where approximately 100 animals less in their herd.

When reading literature about measuring cortisol in hair, it is concluded is that cortisol measures in hair samples correlates with other measurements of cortisol (saliva and feces). Most study's conclusion was that it is a good way of stress measuring even though it needs further investigations (Accorsi *et al.* 2008, Burnett *et al.* 2014). Studies performed on pigs that compared the cortisol levels of healthy and sick animals showed no significant differences (Trevisan *et al.* 2017), while other studies have concluded that clinically diseases in cattle can show differences in cortisol in hair, while subclinical diseases can not (Burnett *et al*. 2015). There is therefore unclear what will affect the cortisol levels in the hair, so over all, cortisol analysis in hair should be more investigated and compared against regularly taken blood/saliva/urine/faeces samples to evaluate further if it is a successful way to investigate chronic stress.

CONCLUSIONS

According to the documentation of cattle loss received from the head of livestock, 136 cattle were killed between June 2015 and November 2016. Lions were documented to be the reason to 57% of all attacks, and most of them occurred during daytime when the cattle were out grazing.

The results in this study indicates that the cattle does not become chronic stressed of a predator attack in the herd.

- There was no significant difference in cortisol levels between attacked and non-attacked animals. There was a statistical tendency (p=0,057) that the attacked Boran cattle had higher levels of cortisol in their hair than the Boran control group.
- No significant differences in cortisol levels in the hair between steers and cows.
- There was also no significant difference in the cortisol amount in the hair between different body conditioning scores of the animals.

There were multiple factors that could affect these results, such as different breeds between cows and steers, the age differences and also that cows and steers grew up in different surroundings.

To get more reliable conclusions, the suggestions would be to collect hair samples from a bigger amount of animals. All animals should be of the same breed and gender, and also of the same age. The control animals should be of the same color as the attacked once, to get as equal color distribution as possible. The hair from the animals should preferably be cut twice to be confident about the time frame of the result.

ACKNOWLEDGEMENT

I would like to devote a big thanks to everyone that helped me along the way with this study, especially my mentor - Jens Jung and Lena-Mari Tamminen and Anna Svensson who helped with the cortisol analysis. Also, I would like to thank Ol Pejeta Conservancy with staff for making this whole study possible.

REFERENCES

Accorsi, P.A., Carloni, E., Valsecchi, P., Viggiani, R., Gamberoni, M., Tamanini, C., Serem, E. 2008. Cortisol determination in hair and faeces from domestic cats and dogs. *General and Comparative Endocrinology,* vol. 155, pp. 398-402.

Amador-Alcalá, S., Naranjo, E.J., Jiménez-Ferrer, G. 2013. Wildlife predation on livestock and poultry: implications for predator conservation in the rainforest of south-east Mexico. *Oryx*, vol.47, pp. 243-250.

Aryal, A., Brunton, D., Ji, W., Barraclough, R.K., Raubenheimer, D. 2014. Human-carnivore conflict: ecological and economical sustainability of predation on livestock by snow leopard and other carnivores in the Himalaya. *Sustainability Science*, vol. 9, pp. 321-329.

Beerda, B., Schilder, M.B.H., Janssen, N.S.C.R.M., Mol, J.A. 1996. The Use of Saliva Cortisol, Urinary Cortisol, and Catecholamine Measurements for a Noninvasive Assessment of Stress Responses in Dogs. *Hormones and Behaviour*, vol. 30, pp. 272-279.

Burnett, T.A., Madureira, A.M.L., Sliper, B.F., Tahmasbi, A., Nadalin, A., Veira, D.M., Cerri, R.L.A. 2015. Relationship of concentrations of cortisol in hair with health, biomarkers in blood, and reproductive status in dairy cows. *American Dairy Science Association*, vol. 98, pp. 4414-4426.

Burnett, T.A., Madureira, A.M.L., Silper, B.F., Nadalin, A., Tahmasbim S., Veiram D.M., Cerrim R.L.A. 2014. Factors affecting hair cortisol concentrations in lactating dairy cows. *Journal of Dairy Science*, vol. 97, pp. 7685-7690.

Comin, A., Peric, T., Corazzin, M., Veronesi, M.C., Meloni, T., Zufferli, V., Cornacchia, G., Prandi, A. 2013. Hair cortisol as a marker of hypethalamic-pituitary-adrenal axis activation in Friesian dairy cows clinically or physiologically compromised. Livestock Science, vol. 152, pp. 36-41.

Dickens, M.J., Delehanty, D.J., Romero, L.M. 2010. Stress: An inecitable component of animal translocation. *Biological Conservation*, vol. 143, pp. 1329-1341.

Edmondson, A.J., Lean, I.J., Weaver, C.O., Farver, T., Webster, G. 1989. A body condition scoring chart for Holstein dairy cows. *Journal of Dairy Science*, vol. 72, pp. 68-78.

Frank, J., Månsson, J., Zetterberg, A. 2016. Viltskadestatistik 2015, Skador av fredat vilt på tamdjur, hundar och gröda. Viltskadecenter. SLU, Grimsö. Riddarhyttan.

Gozález-de-la-Vara, M.R., Valdez, R.A., Lemus-Ramirez, V., Vázquez-Chaqoyán, J.C., Villa-Godoy, A., Romano, M.C. 2011. Effects of adrenocorticotropic hormone challenge and age on hair cortisol concentrations in dairy cattle. *The Canadian Journal of Veterinary Research*, vol. 75, pp. 216-221.

Gow, R., Thomson, S., Rieder, M., Van Uum, S., Koren, G. 2009. An assessment of cortisol analysis in hair and its clinical applications. *Forensic Science International*, vol. 196, pp. 32-37.

Kolowski, J.M., Holekamp, K.E. 2006. Spatial, temporal, and physical characteristics of livestock depredations by large carnivores along a Kenyan reserve border. *Biological Conservation,* vol. 128, pp. 529-541.

Mayer, J.S., Novak, M.A. 2012. Minireview: Hair Cortisol: A Novel Biomarker of Hypothalamic-Pituitary-Adrenocortical Activity. *Endocrinology*, vol. 153.

Mayer, J., Novak, M., Hamel, A., Rosenberg, K. 2014. Extraction and analysis of cortisol from human and monkey hair. Journal of Visualized Experiments, vol. 83, pp. 50882.

Miller, G.E., Chen, E., Zhou, E.S. 2007. If It Goes Up, Must It Come Down? Chronic Stress and the Hypothalamic-Pituitary-Adrenocortical Axis in Humans. *Psychological Bulletin*, vol. 133, pp. 25-45.

Moya, D., Schwartzkopf-Genswein, K.S., Veira, D.M. 2013. Standardication of a non-omvasive methodology to measure cortisol in hair of beef cattle. Livestock Science, col. 158, pp. 138-144.

Möstl, E., Palme, R. 2002. Hormones as indicators of stress. *Domestic Animal Endocrinology*, vol. 23, pp. 67-74.

Novak, M.A., Hamel, A.F., Kelly, B.J., Dettmer, A.M., Meyer, J.S. 2013. Stress, the HPA axis, and nonhuman primate well-being: A review. *Applied Animal Behaviour Science*, vol. 143, pp. 135-149.

O'Brian, K.M., Tronick, E.Z., Moore, C.L. 2012. Relationship between Hair Cortisol and Percieved Chronic Stress in a Diverse Sample. *Stress and Health*, vol. 29, pp. 337-344.

Ogada, M, O., Woodroffe, R., Oguge, N, O., Frank, L, G. 2003. Limiting Depredation by African Carnivores: the Role of Livestock Husbandry. *Conservation Biology,* vol. 17, pp. 1521-1530.

Ol Pejeta Conservancy. 2016. http://www.olpejetaconservancy.org/conservation/boran/

Paszynska, E., Dimitrzak-Weglarz, M., Tyszkiewicz-Nwafor, M., Slopien, A. 2016. Salivary alpha-amylas, secretory IgA and free cortisol as neurobiological components of the stress response in the acute phase of anorexia nervosa. *The World Journal of Biological Psychiatry*, vol, 17, pp. 266-273.

Patterson, B.D., Kasiki, S.M., Selempo, E., Kays, R.W. 2004. Livestock predation by lions (Panthera leo) and other carnivores on ranches neighboring Tsavo National ParkS, Kenya. Biological Conservation, vol. 119, pp. 507-516.

Peric, T., Comin, A., Corazzi, M., Montillo, M., Cappa, A., Campanile, G., Prandi, A. 2013. Hair cortisol concentrations in Holstein-Friesian and crossbreed F1 hiefers. American Dairy Science Association, vol. 96, pp. 3023-3027.

Peters, H.F., Slen, S.B. 1964. Hair coat characteristics of bison, domestic X bison jubris, cattalo, and certain domestic breeds of beef cattle. *Canadian Journal of Animal Science*, vol. 44.

Phillips, C. J. C. 2010. Principles of cattle production. Second edition. Cab international, United Kingdom, pp. 57.

Rushen, J., Passillé, A.M., von Keyserlingk, M.A.G., Weary, D.M. 2008. Animal Behaviour. In Phillips, C, (Edit), The Welfare of Cattle, vol. 5, The Netherlands: Springer, pp. 70-11.

Thorn, M., Green, M., Dalerum, F., Bateman, P.W., Scott, D.M. 2012. What drives human-carnivore conflict in the North West Province of South Africa? *Biological Conservation*, vol. 150, pp. 23-32.

Trevisan, C., Montillo, M., Prandi, A., Mkupasi, E.M., Ngowi, H.A., Johansen, M.V. 2017. Hair cortisol and dehydroepiandrosterone concentrations in naturally *Taenia solium* infected pigs in Tanzania. *General and Comparative Endocrinology*, vol. 246, pp. 23-28.

Wosu, A.C., Valdimarsdóttir, U., Shields, A.E., Williams, D.R., Williams, M.A. 2013. Correlates of Cortisol in Human Hair: Implications for Epodemiologic Studies on Health Effects of Chronic Stress. *Ann Epodemiol*, vol. 23, pp. 797-811.