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Predator Recognition Behaviors and Stress Hormones in an Endangered Captive Mammal: Implications for Reintroduction

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Predator Recognition Behaviors and Stress Hormones in an Endangered Captive Mammal: Implications for Reintroduction

by

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ABSTRACT

One purpose of captive breeding programs for endangered species is the potential reestablishment of wild populations. However, behavioral problems resulting from relaxed selection or adaptation to captivity can lead to decreased predator recognition and increased mortality in reintroduced individuals. Predator training of prey animals can reduce this mortality, but a species must have some instinctual response to the signs of predators as a prerequisite for successful training. The Tadjik markhor (*Capra falconeri heptneri*) is one of the most endangered mammals in the world and may be reintroduced to portions of its former range in the future. We assessed the potential of captive Tadjik markhor to recognize signs from their natural predators using visual and olfactory cues, and compared their behavior and stress hormone levels to baseline levels and to novel but non-threatening cues. Mean percent time in vigilance behavior did not differ between predator and control cues, but both were higher than baseline (ANOVA; $P < 0.001$). However, markhor exhibited more alarm calls and ear flicks when faced with predator cues than when faced with control cues or during baseline observations (MRPP, $P < 0.001$). We found no difference, however, in fecal glucocorticoid (GC) levels among the three treatment types. These results suggest that captive markhor have not entirely lost their ability to recognize threats from potential predators and may respond to pre-release training in the event of a reintroduction program that uses captive-raised individuals.

Keywords: Anti-predator behavior, *Capra falconeri heptneri,* Glucocorticoids, Predator Recognition, Reintroduction, Tadjik Markhor

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Lastly I would like to thank my late doggy Tasha. Everyday coming home from work or school she greeted me with her smiling face and wagging tail. Her unconditional love made every single day better.

INTRODUCTION

Animal reintroductions attempt to reestablish populations of species that are extinct in the wild or extirpated from portions of their former range (Armstrong & Seddon 2008; Seddon et al. 2007). Reintroductions have seen increased use in recent decades and zoo-based captive breeding programs are increasingly being looked to for use in reintroductions to conserve threatened species. To prevent their extinction, thousands of species of vertebrates may need to be bred in captivity for the purpose of eventual reintroduction (Soulé et al 1986). Yet reintroduction programs typically have low success rates (Armstrong & Seddon 2008). Much of this failure is due to the fact that animals raised in a captive environment can differ significantly in their behavior from their wild counterparts (Mathews et al 2004).

Wild animals must use multisensory perception to successfully navigate their environment to find food, mates, and other resources and to avoid predation (Munoz & Blumstein 2004; Sih1992). Yet reintroduction programs aiming to restore populations of endangered animals often use individuals that are captive-bred and therefore also often lack the behaviors needed to survive in the wild. Much of the mortality of reintroduced individuals is from predation because introduced animals lack the ability to both recognize potential predators and express appropriate anti-predator behavior (Beck 1991; Griffin et al. 2000). In most cases of high mortality resulting from predation, the captive-reared animals had no experience dealing with predators before release (Griffin et al. 2000: McLean et al. 1995). Important behaviors for survival in natural environments may be lost due to relaxed selection caused by the isolation from predators or a change in the direction of selection to adapt to the captive environment (McPhee 2003). Generally, as the number of generations in captivity increase, so does the variance in behaviors that would help an animal recognize and avoid predation (McPhee 2003). Similar findings have been reported on the behavior of wild animals that have been subject to relaxed selection by being isolated from predators for long periods of time due to extinction or extirpation (Blumstein et al 2004; Blumstein and Daniel 2005; Lahti et al. 2009). Griffin et al. (2000) concluded in a review of the use of training as a method to improve survival in reintroduced animals that those that experienced ontogenetic rather than evolutionary isolation would fare better upon release. The zoo or captive setting would be classified as evolutionary isolation. The authors also concluded that pre-release training is likely to be unsuccessful for animals that do not display any precursors to anti-predator behavior. It may then be useful to test, while still in an ordinary captive zoo setting, the extent to which threatened species recognize cues from their potential predators.

With this study we aimed to determine whether captive-bred Tadjik markhor (*Capra falconeri heptneri*) have retained the ability to recognize cues signifying the presence of natural predators. Markhor are one of the most endangered mammal species and the Tadjik subspecies is considered critically endangered, with likely fewer than 700 remaining in the wild (LaBarge 2012; Valdez 2008). Because there are so few remaining Tadjik markhor, a reintroduction program may someday be established in cooperation with the Association of Zoos and Aquariums (AZA) (LaBarge 2012; Pokoradi 2005). Determining whether markhor can recognize and respond to their natural predators may then be essential for their future survival as a species.

The Tadjik markhor (also known as the Turkmenian Markhor) is native to Turkmenistan, Tajikistan, Uzbekistan, and possibly Northern Afghanistan, where it exists in small and scattered populations. Their main threats to survival have been poaching for traditional Asian medicines and meat, habitat loss, competition and introduced diseases from grazing agricultural animals, and war (Michel et al. 2014; Valdez 2008). Several conservation measures for the Tadjik markhor, including ex-situ breeding programs for possible reintroduction, have been implemented to prevent their extinction (LaBarge 2012). In North

America, all markhor housed in AZA-accredited zoos likely belong to the Tadjik subspecies (Hammer et al. 2008; LaBarge 2012).

Markhor have not been well studied in the wild or in captivity but other species in the genus Capra have been studied for their vigilance behaviors in response to predation and other stressors. Wild Ibex typically stand and look intently at their surroundings and direct their ears forward, alarm call, and flee when a predator is spotted (Alados & Escos 1988; Hochmen & Kotler 2006).

There were no previous studies that addressed the hormonal stress response of captive or wild markhor but other captive goats, especially in agriculture, have been studied to determine hormonal responses to fear or stress-provoking stimuli. Glucocorticoid (GC) levels in domestic goats do not differ by sex or time of day. However there is evidence that photoperiod and seasonality do influence GCs (Fazio et al. 2006). This may be true for markhor as well because they may be one of the most closely related remaining wild relatives to domestic goats (Hammer et al. 2008). In other zoo-housed species GC analysis is used as an important indicator of chronic stress and welfare (Millspaugh & Washburn 2004; Wielebnowski et al. 2000). Elevated cortisol levels approximately 12 hours after exposure to these cues could also indicate stress due to predator recognition (Boiski et al. 1999; Barcellos et al. 2007; Fazio et al. 2006; Woodley & Peterson 2003).

If captive markhor have retained the ability to recognize predators, they should also show an increase in alertness and vigilance behaviors and a decrease in resting and foraging behaviors in the presence of cues from their natural predators. Our objective was to compare changes in behavioral and hormonal stress responses after exposure to predator cues and novel but non-threatening cues. Our results will help to determine whether a reintroduction using captive-bred individuals would be appropriate for this species.

METHODS

Study Species

The focal population for this study consisted of five Tadjik markhor housed at the Rosamond Gifford Zoo of Syracuse, New York. This herd consisted of two adult females, one adult male, one sub-adult female, and one sub-adult male (Table 1). All markhor in North American AZA zoos likely belong to the Tadjik (*C.f. hepneri*) subspecies (Hammer et al. 2008). Those previously listed as "unclassified" have been corrected to be part of this subspecies by mtDNA analysis (LaBarge & Schad 2012). The 17 "founders" of the North American zoo population came from the Moscow Zoo Center in Russia between the years 1960-1975. Whether those individuals were captive-bred or captured from the wild is unknown, so the number of generations in captivity is unknown (LaBarge 2012).

In the wild, Tadjik markhor are most often preyed upon by grey wolves (*Canis lupus*), snow leopards (*Panthera unica*), and the Eurasian lynx (*Lynx lynx*), which generally only takes young animals (Michel et al. 2014). In captive-bred animals, signs of the presence of these predators should produce a stressed behavioral response if predator recognition is instinctive, whereas exposure to novel, non-predatory cues should not cause a stress response (Blumstein et al. 2000, Blumstein et al. 2006; Griffin et al. 2001; Sündermann et al. 2008).

Keepers at the Rosamond Gifford Zoo have noticed that under stressful conditions markhor will tilt their heads back. Usually this behavior is accompanied by elevating the front two limbs on a boulder or the sides of the fence that surrounds their exhibit. Other behaviors that potentially indicate stress include tilting the ears forward and alarm calling. These behaviors could therefore be indicative of stress from a fear of predators, if captive markhor were exposed to predator-related cues.

Study Area

This study was conducted at the Rosamond Gifford Zoo in Syracuse, New York. The exhibit is approximately one half acre of steep, rocky terrain with grass near the public viewing area. In the morning keepers provide pellets in food dishes but hay and grass as well as foliage from trees and shrubs are available and eaten throughout the day.

Observation Methods

We observed individual markhor in their enclosures from 22 May 2013 to 5 September 2013 and from 11 May 2014 to 8 September 2014 using focal animal sampling (Altmann 1974) with a total of 83 observations. Individuals were watched for 40 minutes, with behavior and location recorded every two minutes. Time-sampled behavior included resting, eating, drinking, sparring, scratching, standing, walking, and climbing. Continuously recorded (tallied) stress responses included the head-tilt, tilting the ears forward, and alarm calling.

Observation Cues

We placed predator cues just outside the exhibit but in view of the markhor at the beginning of nine observations, including a mounted Canada lynx (*Lynx canadensis*), scent from grey wolves (*Canis lupus*), and scent from snow leopards (*Panthera unica*). Both of these scents were from enrichment items placed in wolf and leopard exhibits that keepers observed the carnivores using frequently. We used a mounted turkey (*Meleagris gallopavo*) and vanilla scent in six observations to control for reactions to novel visual and olfactory stimuli.

Fecal Sample Corticosteroid Extraction

We collected fecal samples from individual markhor approximately 12 hours after the beginning of a behavioral observation. All samples were collected at approximately the same time of day (late afternoon/evening) to control for changes due to circadian rhythms. The samples were then stored at -10˚C until they were shipped overnight to the Smithsonian National Zoo Department of Reproductive Endocrinology. The samples were later freeze-dried and 1.0 gm of each was weighed out for extraction in 16 X 125 glass tubes. Five milliliters of 90% ethanol and 100µl of cortisol tracer were added to each individual sample tube. The samples were then centrifuged at 1500 rpm and decanted into another set of glass tubes with 5ml of 90% ethanol. Each sample was then vortexed for 30 seconds. The samples were then centrifuged again for 20 minutes and then added to the remaining supernatant. The supernatant was then diluted with water to a ratio of 1:10 and run on a cortisol enzyme immunoassay (EIA).

Data Analysis

Two-sample t-tests were used to compare mean proportions of time spent in "vigilant" behaviors to time spent in "non-vigilant" behaviors within baseline, control cue, and predator cue observations. The behaviors "resting," "eating," "drinking," "sparring," and "scratching" were considered non-vigilant. The behaviors "standing," "walking," and "climbing" were all considered "vigilant."

We used one-way ANOVA to test if the mean proportion of time spent in vigilant behaviors was different in observations with a predator cue from the baseline observations and observations with a control cue. Tukey's HSD *post-hoc* test was used for multiple comparisons. We used individuals as blocks to account for heterogeneity among animals. The residuals conformed to the assumptions of ANOVA. We analyzed continuously recorded behavioral data using Multiple Response Permutation Procedure (MRPP) to test if the median number of stress response counts differed between treatments, using Blossom (Cade & Richards 2001). This test is a nonparametric analog to MANOVA and compares the distributions of observations among categories, and provides an exact P-value for small sample sizes. We summarized these distributions using the Multivariate Medians and Distance Quartiles (MEDQ) module in Blossom. We considered results to be significant at an α level of 0.05, and used a Bonferonni correction when performing *post-hoc* multiple comparisons using MRPP. We calculated the mean GC level for each individual markhor along with the standard error for for all three treatment types.

RESULTS

Behavioral observations

During baseline observations, individual markhor spent significantly more time in non-vigilant than in vigilant behaviors (T_{106}=-8.69, P<0.001). There was no difference between time spent in vigilant and non-vigilant behaviors in predator cue observations (T_{15}=-0.65, P=0.527) or control cue observations (T_9=-1.11, P=0.295).

The proportion of time allocated to vigilant behaviors differed among the three treatment types (Figure 1). Markhor spent a lower proportion of time in vigilant behavior during baseline observations than during predator- or control-cue observations (Figure 1). However, there was no difference between predator- and control-cue treatments.

The number of stress responses per 40-min trial differed among the observation categories (Table 2). The median number of alarm calls and ear flicks per individual were greater during predator treatment observations than the other two treatments (P < 0.001, Table 2). However, head tilt behavior did not differ among the three treatments (P=0.171, Table 2).

Glucocorticoid Results

There was no difference in the mean level of GCs for the baseline, predator cue, or control observations per individual animal (Figure 2.).

DISCUSSION

Our results suggest that captive markhor at the Rosamond Gifford zoo have not entirely lost their ability to recognize predators and that this ability is also at least partially independent of experience. Markhor responded differently to the predator cue observations than the novel non-threatening cue (control) observations or the baseline. Markhor responded to signs from predators by increasing the number of alarm calls and ear flicks but did not show the characteristic stress behavior of tilting their head all around. This may indicate that the head tilt behavior is mainly a result of stress from the captive environment (e.g veterinary procedures, stressors from keepers and visitors).

Time spent in vigilant behaviors did not differ between predator cue and non-threatening novel cue observations, although in both treatment categories vigilant behavior was elevated over baseline levels. Similarly, Blumstein et al. (2000) found that wild Tammar wallabies (*Macropus eugenii*) that experienced isolation from predators responded to visual cues of potential predators by changing their activity and increasing vigilance and decreasing foraging behaviors even after cues were removed. Moreover, Hotchman and Kolter (2006) found that wild Nubian ibex (*Capra nubiana*) responded to potential threats by changing their foraging behavior and increasing active vigilance and scanning behaviors. Our results suggest that markhor may become more vigilant when any type of novel cue is presented even if it is nonthreatening, but do not exhibit stress response unless the cue is evaluated as a danger. In contrast, Monclus et al. (2005) found that predator-naïve European rabbits (*Oryctolagus cuniculus*) had an innate ability to recognize signs from predators but did not change their activity budget or foraging behavior.

The results from the GC analysis suggest that, physiologically, individual markhor were not significantly stressed by predator cues compared to control cues or the baseline observations. However, there are many variables that can influence or confound analyzed GC levels (Millpaugh & Washburn 2004). In this study we were able to only collect fecal samples approximately 12 hours after a behavioral observation while other studies suggest that, in some mammals, fecal GC levels are highest at or after 24 hours past a stressful event (Smith et al. 2012). Space and food availability and interactions with other animals in the captive environment can influence stress hormone levels (Wielebnowski et al. 2002). In addition, because fecal GC metabolites reflect an average amount of hormone over a certain period of time they do not necessarily reflect spikes at certain points as blood samples can (Harper & Austad, 2000; Millspaugh & Washburn 2004).

One motivation for beginning this research was that the Association for Rescue of Endangered Species of Animals reportedly initiated a captive breeding program in the Lesser Carpathians of Slovakia in the early 2000s with the aim of eventually reintroducing young adult markhor into the wild (Pokoradi 2005). However, this organization has not released any further reports. Because of how endangered the Tadjik markhor is presently, a reintroduction program in cooperation with European and North American zoos may someday have to be established to keep this subspecies from going extinct. Therefore, it is important that we gain as much information as possible about the current behaviors and possible behavioral deficiencies of captive individuals.

Based on the results from this study, captive markhor appear to have some instinctual response to cues from predators and are not simply responding to novel stimuli. This is a prerequisite for any predator-training program for species that are predator naïve and going to be released (Griffin et al. 2000). Because of the threats facing wild markhor and because captive markhor have been able to retain these behaviors that are important for survival in the wild, this species might be targeted within zoos as a higher priority for strategic breeding. Given their greater proximity to the native range of the Tadjik markhor, European zoos might be especially well suited to do this.

CONCLUSION

Although there is no known planned reintroduction of markhor in cooperation with AZA zoos, it is important to determine before any program takes place whether training animals to avoid predation might be effective. The findings of this study suggest that, after many generations in captivity, the critically endangered Tadjik markhor has retained the ability to recognize cues from potential predators. Further research should address the behavioral suitability of other threatened species held within zoos to survive in the wild as well as differences in individual animals. Further studies might also benefit from determining whether animals respond differently single modality cues versus multimodal cues (i.e., different senses from the same source) like those they might experience in the wild (Ward & Mehner 2010).

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Table 1. Herd composition of markhor for two seasons of data collection (May-September).

Year	Individual	Date of Birth	Sex
2013	Sunny	5/2010	M
	Edith	5/2010	F
	Marisa	7/2012	F
	Thor	5/2011	M
	Drizzle	5/2010	F
2014	Thor	5/2011	M
	Drizzle	5/2010	F
	Marisa	7/2012	F
	Turgan	6/2013	M
	Sasha	6/2013	F

Table 2. Median number of markhor stress response counts per 40 minute observations, for each treatment type.

		Alarm Call			Head Tilt			Ear Flick		
Treatment	n	Median [a]	Q1	Q3	Median	Q1	Q3	Median	Q1	Q3
Baseline	68	0.0 B	0.0	0.0	0.00 B	0.0	0.0	0.0 B	0.0	0.0
Control	6	0.0 B	0.0	0.0	0.00 B	0.0	0.0	0.0 B	0.0	0.0
Predator	9	0.8 A	0.0	1.0	0.40 B	0.0	1.0	2.2 A	0.0	4.0

[a]Within stress response categories, medians with the same capital letter are not significantly different (Multiresponse Permutation Procedure, Pearson Type III test statistic = -13.2, P<0.001). 25th percentile (Q1) and 75th percentile (Q3) are shown.

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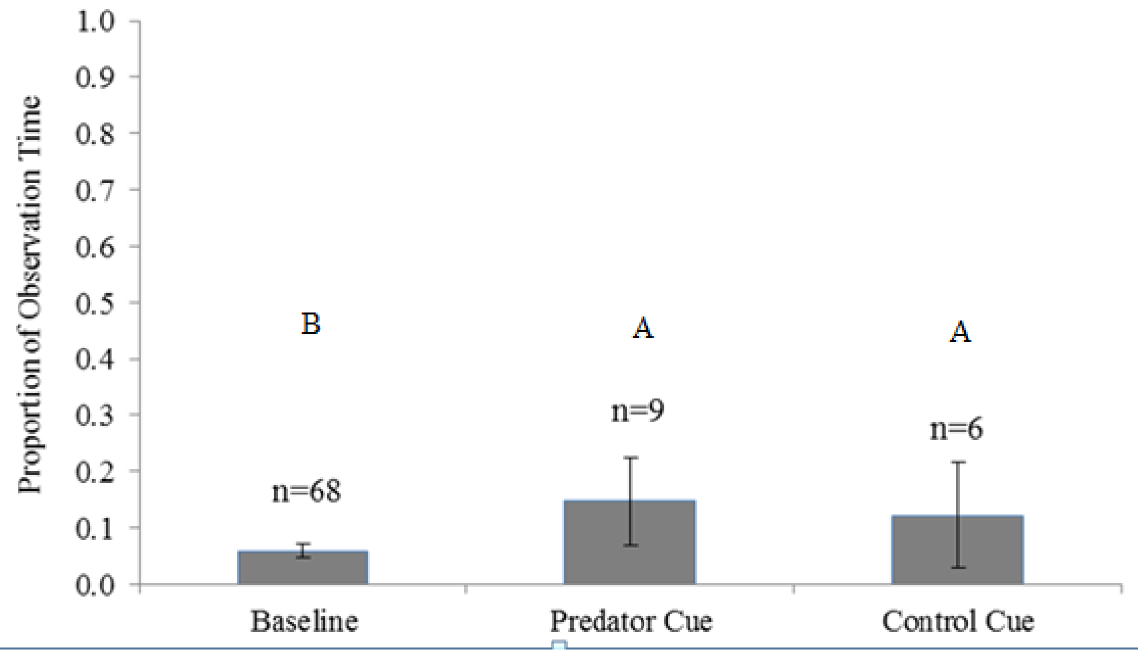

Fig. 1. Mean proportion of observation time (40 min) spent in vigilant behaviors by markhor for three treatment types. SE bars shown. Means with the same capital letter are not significantly different (ANOVA, $F_{2,82}$=10.93, < 0.001).

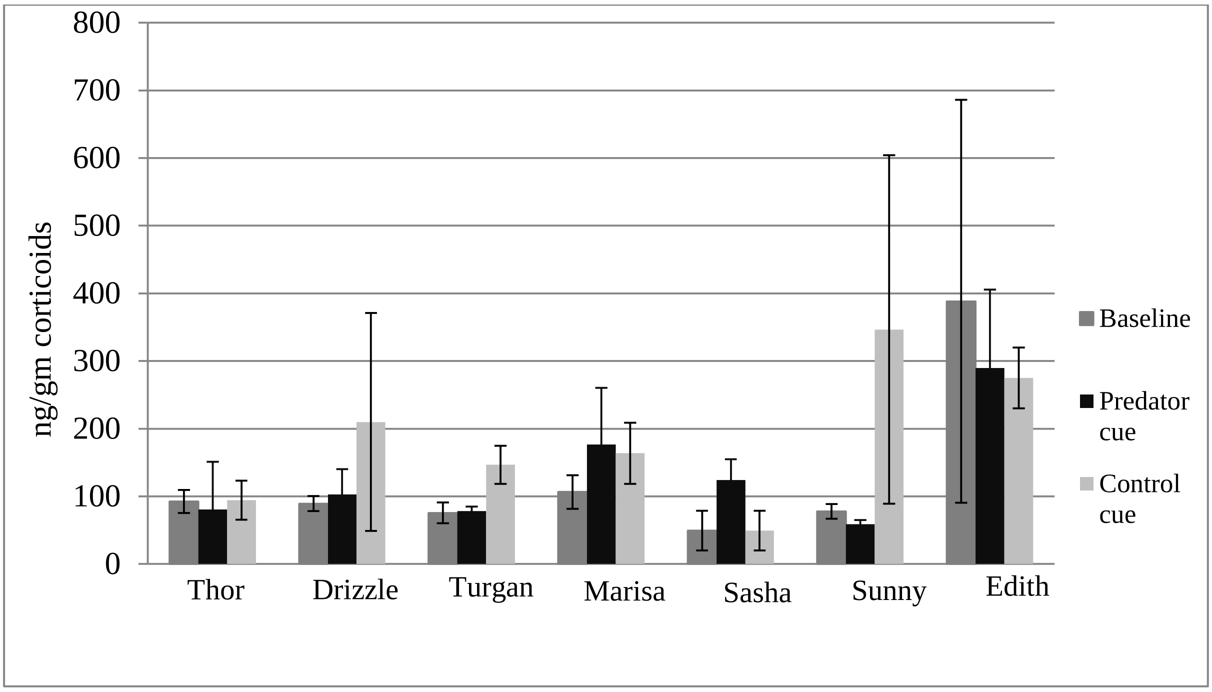

Fig 2. The mean concentration (nanogram per gram) of glucocorticoids in fecal samples after baseline, predator cue, and control cue observations. SE bars shown.