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Impacts of Livestock Grazing on Fecal Glucocorticoid Levels and Gastrointestinal Parasite Prevalence in Blue Sheep in Spiti Valley, Western Himalayas

Priya Iyer[1], Vinod Kumar[2], Mahender Reddy[2] and Govindhaswamy Umapathy[2*]

[1]Centre for Ecological Sciences, Indian Institute of Science, Bangalore – 560012, Karnataka, India
[2]Laboratory for the Conservation of Endangered Species (LaCONES), CSIR-Centre for Cellular and Molecular Biology, Hyderabad – 500007, Telangana, India; guma@ccmb.res.in

Abstract

Livestock grazing in protected areas is known to affect wild species. In this study, we examined fecal Glucocorticoid Metabolite Concentration (fGCM) and gastrointestinal parasite prevalence in blue sheep populations of Spiti valley, Himalayas. We collected 156 fecal samples of blue sheep from areas under intense livestock grazing and areas without small livestock during 2014-15. We also collected 27 fecal samples from livestock to examine parasite prevalence. We found that blue sheep in livestock-grazed areas had higher fGCM than non-grazed areas. Similarly, parasite prevalence was higher in areas with presence of cattle. Overall, adult females were more affected by disturbance and grazing compared to males and young ones. This is the first study to document the physiological stress response of wild ungulates to livestock grazing in Indian sub-continent. We suggest management recommendations to set aside pastures for exclusive use by wild blue sheep, regularly deworm livestock, and decrease the stray dog population.

Keywords: Blue Sheep/Bharal (*Pseudois nayaur*), Fecal Glucocorticoid Metabolites, Livestock Grazing, Parasite Prevalence, Trans-Himalayas

1. Introduction

Spiti valley is located in the Indian Trans-Himalayas, in the state of Himachal Pradesh and forms an edge of the Tibetan plateau (Figure 1). It falls in the rain-shadow region of the Great Himalayas and is a high altitude, cold desert. Hence, the climate is arid, and the vegetation is sub-Alpine scrub meado[1,2]. The landscape is characterized by a lack of trees, and is instead dominated by grasses, sedges and few shrubs. Most of the precipitation occurs as winter snow and the region receives almost no rain from the Indian monsoon. The wildlife present in the area are adapted to the cold climate and high altitude. Large mammals in the area include the charismatic and endangered snow leopard, blue sheep (also called bharal), ibex and wolves. The region also hosts red foxes, woolly hares, pika, marmots, voles and many birds including the raptors Himalayan griffon, golden eagle and lammergeier. Due to overstocking of domestic herbivores in this region, many wild herbivores such as Argali, Chiru, Kiang and wild yak may have become extinct locally[3]. The region has been inhabited by pastoral people of Tibetan origin

**Author for correspondence*

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over two millennia[4]. The high altitude pastures provide nutritious grass for domestic ungulates, and hence people have traditionally reared sheep, goats, cows, yaks, yak-cow hybrids, donkeys and horses in the area.

The impacts of the human disturbance on the wild animals in this landscape have been studied in detail[1,5–7]. These rangelands are overstocked, with 10 times more domestic herbivores than wild herbivores[5]. Livestock populations are maintained at artificially high levels through supplementary feeding in winter. Goats and sheep may exclude ibex from habitat patches[6], and the forage available for bharal is significantly reduced by livestock grazing[8]. As a consequence, bharal density as well as population performance (in terms of kids per female) are significantly reduced in intensely grazed pastures[7,8]. These changes are expected to have significant impact on the population and behavior of the apex predator, the snow leopard, and it might also increase the human-wildlife conflicts in the region[2]. While the studies above have considered the impacts of livestock on vegetation and wild herbivore population performance, no study has yet considered the physiological and parasite load impacts of the co-existence of livestock and wild herbivores in Spiti valley.

Many studies have reported increased cortisol levels in wild animals due to human activities- e.g., snow-mobiles increased stress in wolves and elk[9], ski tourism increased stress in Capercaillie[10], snow sports in black grouse[11], anthropogenic stressors including state holidays in coyotes[12], roads in elks[13], habitat disturbance in owls[14] and human encounter rates and distance to roads in tigers[15]. There are very few studies on stress impacts of cattle grazing on wild herbivores, e.g., evidence that wood mice are affected by presence of cattle[16], but a negative result regarding the impact of cattle grazing on Algerian mice[17]. This study aims at understanding the impacts of livestock grazing on blue sheep (*Pseudois nayaur*) with reference to physiological stress (fecal glucocorticoid metabolite concentration) and parasite prevalence.

2. Methods

2.1 Study Areas

The study was conducted in five locations viz Kibber, Chunkar, Lari, Tashigang and Lobdur in Spiti valley of Himachal Pradesh, India (Figure 1). The people in all villages are predominantly agro-pastoralists (growing green peas and barley), with some families also involved in tourism[2].

Kibber is a village of about 70 households located at an altitude of 4200 m. The villagers own a variety of livestock (approximate numbers for the entire village in

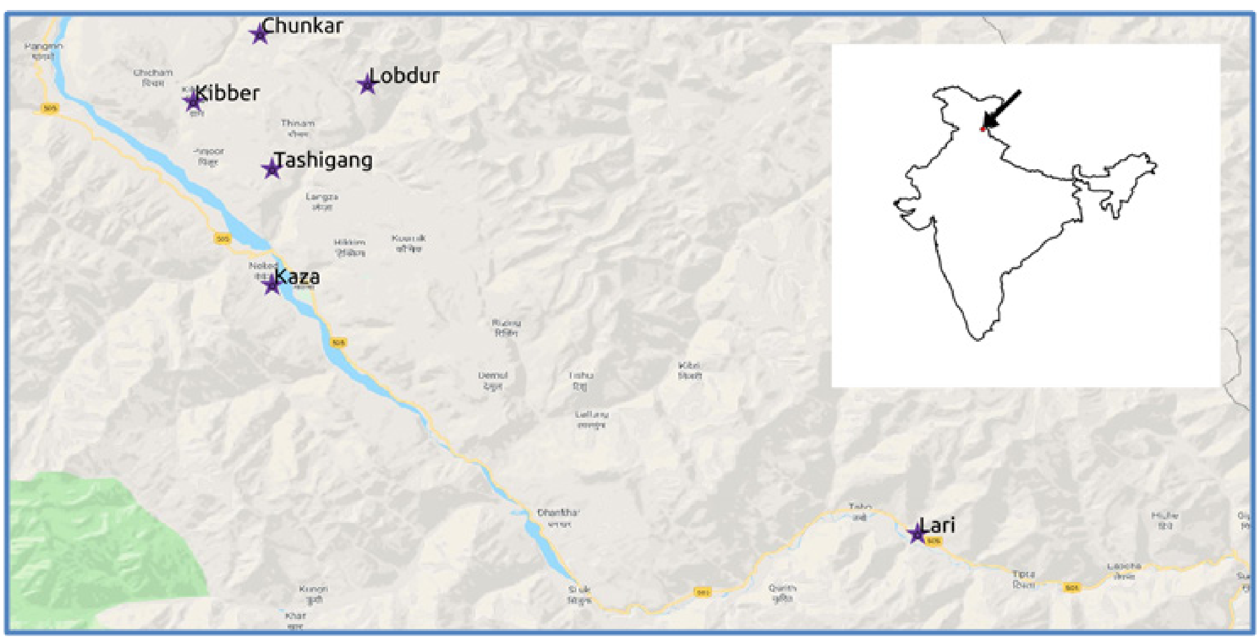

Figure 1. Map of Spiti with 5 study locations.

parentheses): sheep and goats (400), cows (50), yaks (210), yak-cow hybrids (140), horses (11) and donkeys (160). The pastures around Kibber are grazed by both blue sheep and domestic animals. Chunkar (4300 m) is a protected Reserve located about 6 km from Kibber. Villagers do not allow their livestock to enter the Reserve. However, free-ranging horses and yaks may use this pasture occasionally. Blue sheep are expected to move regularly between Chunkar Reserve and the Kibber pastures.

Lari, located at 3300 m altitude, is the lowest-altitude study area. It consists of about 50 households, most of whom have switched to apple cultivation since the past 8-10 years. They also stopped keeping goats and sheep since that time. Lari villagers keep cows (70), yak-cow hybrids (17) and donkeys (10). The pastures around Lari are co-grazed by blue sheep and larger livestock.

Tashigang is one of the highest villages in Spiti, at the height of 4500 m. It consists of only 5 households, who keep cows (21), yaks (35), yak-cow hybrids (7) and donkeys (1). Lobdur (4600 m) is another grazing Reserve, located 9 km from Tashigang (4500 m). Tashigang villagers do not keep any goats and sheep, and their larger cattle are not allowed into the Reserve at Lobdur. Villagers report that the blue sheep herds in Lobdur stay in the Reserve and do not mix with the herds around Tashigang.

Snow leopards are frequently sighted around Kibber, Chunkar, Tashigang and Lobdur, and less frequently around Lari. However, Lari may have occasional visits of wolves. We collected blue sheep fecal samples from Kibber, Chunkar and Lari in August-September 2014, and analyzed them for fGCM concentrations and parasite prevalence. We also collected fecal samples from domestic goats and sheep from Kibber during 2014. We collected blue sheep fecal samples from Tashigang and Lobdur in August 2015 and analyzed them for fGCM concentrations.

2.2 Sample Collection Methods

Before sampling, we spotted blue sheep herds using binoculars (10x50) and approached them to distances of 20-100 meters (as permitted by the terrain) without disturbing the herd. Following spotting a herd, we recorded the number of animals and age/sex composition of the herd[8]. We then observed the individual animals with binoculars until they defecated, and noted the site of their defecation with respect to other landmarks in the area (such as distinct rocks or plants/shrubs). Once the herd moved away from this area, we collected the fecal samples from each location. We further divided each sample into two portions, one for fecal hormone extraction and the other for parasite screening. For parasite analysis, the samples were stored in 10% formalin solution and transported to the LaCONES-CCMB, Hyderabad, for further analysis. Overall, we collected 156 fecal samples from blue sheep (Table 1) and 27 fecal samples from goats and sheep from Kibber village during the study period.

Table 1. Details of sample collection locations, livestock, human settlements and mean fGCM concentrations

Location	Human settlement at location	Altitude in m	Livestock	Number of bluesheep samples	Mean fGCM concentration ± standard error (ng/g)
Kibber	Yes	4200	Goats, Sheep, Cows, Yaks, Yak-Cow hybrids, Horses, Donkeys	33	28.6±1.67
Chunkar reserve (protected area)	No	4300	Horses, Yaks (occasional)	18	25.6±3.96
Lari	Yes	3300	Cows, Yak-cow hybrids, Donkeys	26	11±2.44
Tashigang	Yes	4500	Cows, Yaks, Yak-Cow hybids, Donkeys	35	30.7±2.98
Lobdur reserve (protected area)	No	4600	Horses, Yaks (occasional)	44	23.2±2.85

2.3 Parasite Screening

We recorded gastrointestinal parasites using floatation and sedimentation techniques[18]. We identified helminth eggs, larvae and protozoan cysts in fecal samples based on their morphological traits such as size, wall structure, internal content and shape[19]. We defined the prevalence of parasite as the percentage of samples with a given faunal taxa[20].

2.4 Extraction of Cortisol Metabolites

Fecal glucocorticoid metabolites were extracted using previously described procedure[21,22]. The dried fecal samples were pulverized, sieved and approximately 0.2 g of fine fecal powder was weighed, boiled in 5 mL of 90 % ethanol for 20 min. The fecal extracts samples were centrifuged at 500 g for 10 min, the supernatants were transferred in fresh tubes and the pellet was resuspended in 5 mL of 90 % ethanol. The samples were vortexed for 1 min, centrifuged, pooled with previous supernatants, dried at 40 ºC, resuspended in 1 mL of absolute methanol and used for Enzyme Immunoassay (EIA).

2.5 Cortisol Enzyme Immunoassay (EIA)

Fecal cortisol concentration was measured using polyclonal cortisol antibody (R4866, kind courtesy Dr. Coralie Munro, University of California, Davis, CA, USA). The cortisol antibody showed cross-reactivity with cortisol 100 %, prednisolone 9.9 %, prednisone 6.3 %, cortisone 5 % and <1 % with corticosterone, desoxycorticosterone, 21 desoxycortisone, testosterone, androstenedione, androsterone and 11-desoxycortisol[23,24]. The cortisol EIA was performed as described previously[23,25]. The assay sensitivity was calculated at 90 % binding and found to be 1.95 pg/well. Parallelism was performed by demonstrating parallel displacement curves between the serial dilution of pooled fecal extracts and the respective standards[23].

2.6 Statistical Analysis

We carried out pairwise comparisons of samples from different locations using Mann-Whitney U tests. We used R Studio and SPSS 17.1 to carry out the statistical analyses.

3. Results

3.1 Fecal Glucocorticoid Metabolites (fGCM)

We found that blue sheep in Kibber had significantly higher fGCM (mean = 28.6±1.67 ng per gram of fecal matter) than blue sheep in Lari (mean = 11±2.44 ng/g; Mann-Whitney U test: W = 758, p = 0.004; Figure 2). This is expected, as Lari has population of sheep and goats. Lari's blue sheep population also had significantly lower fGCM than any other no small livestock compared to Kibber's large location (Mann-Whitney U tests: Tashigang: W = 796, p < 0.0001; Chunkar: W = 399.5, p < 0.0001; Lobdur: W = 593, p < 0.0001). We found that the fGCM concentrations significantly varied among the five locations (Kruskal-Wallis test: X^2 = 49.618, df = 4, p < 0.0001).

The fGCM levels did not differ significantly between Tashigang and the reserve near it, Lodbur (W = 361, p = 0.1149) or between Kibber and the reserve near it, Chunkar (W = 385.5, p-value = 0.0822). However, when these samples were pooled as grazed and non-grazed, we found a significant increase of fGCM in grazed habitats (Kibber and Tashigang: mean = 29.65±1.72 ng/g) compared to the non-grazed reserves (Chunkar and Lobdur: mean = 25.16±2.30 ng/g; Mann-Whitney U test: W = 1921.5, p = 0.022, Figure 3).

We also looked at variation in fGCM among the age-sex classes within populations. We found that Adult Females (AF) had higher fGCM concentrations (mean = 25.18±2.37 ng/g) than adult males (AM; mean = 21.88±1.55 ng/g) in most locations (Figure 4), and these differences were statistically significant for Kibber (M-W U test: p = 0.01) and for Tashigang (p = 0.011). The exception to this pattern is Lari, where both male and female cortisol levels each have one outlier. Even in Lari, AF have higher fGCM than AM if the outliers are removed. Further, AF also had higher fGCM concentrations than young in most locations (Figure 4), and these were statistically significant in Lobdur (M-W U test: p = 0.0048) and Kibber (p = 0.0611).

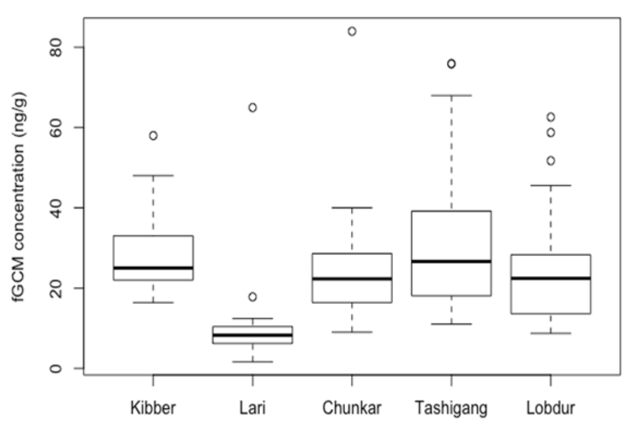

Figure 2. Box-plot showing fGCM concentrations in blue populations of five study areas.

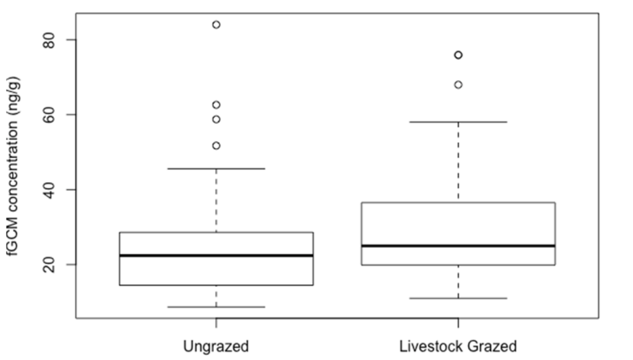

Figure 3. fGCM concentrations in livestock-grazed (Kibber + Tashigang) areas versus ungrazed Reserves (Chunkar + Lobdur).

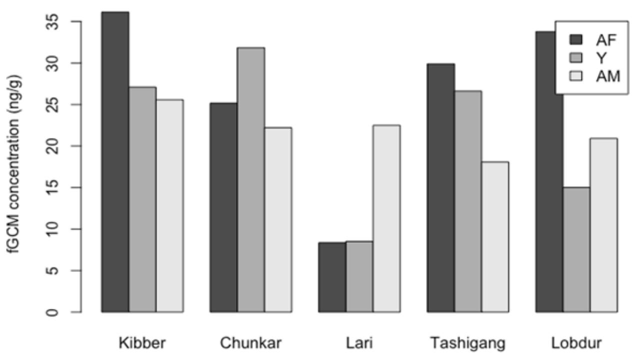

Figure 4. fGCM concentrations among age-sex classes of blue sheep populations in the five study areas

We also found that Lari adult females had the lowest fGCM concentration (mean = 11±2.44 ng/g) among adult females of all the locations (Figure 5) and the comparisons are statistically significant (M-W U test comparisons with Kibber: W = 104, p = 0.0001, Chunkar: W = 65, p = 0.0015, Tashigang: W = 78, p = 0.0007, Lobdur: W = 154, p = 0.0003). Further, Lari young individuals also had lower fGCM than young in most other locations (M-W U test comparisons with Kibber: W = 70, p = 0.00075, Chunkar: W = 32.5, p = 0.0183, Lobdur: W = 49, p = 0.0176).

3.2 Parasite Prevalence

Overall we recorded six parasite taxa consisting of four nematodes (Ascaris, Trichuris, Strongyloides,

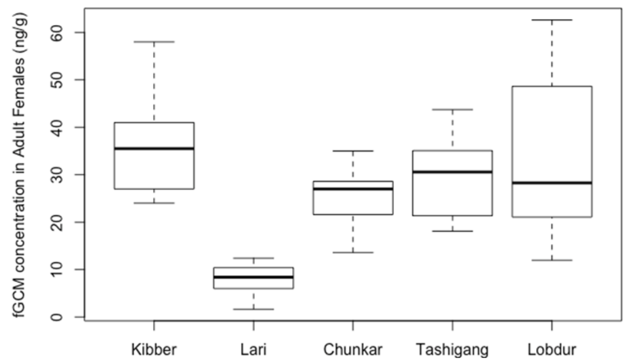

Figure 5. Box plot showing fGCM concentrations in adult females in the five locations

Trichostrongylus), one cestode (Moniezia) and one protozoon (Coccidia). Kibber had the highest number of parasite taxa (all six parasites) and Chunkar Reserve had the lowest with one parasite (Table 2). Similarly, Kibber had the highest parasite prevalence (with 87.8% of the samples infected) followed by Lari, and then Chunkar Reserve (65.4% and 50%, respectively). The livestock samples collected from Kibberhad all the 6 parasite genera with 81.5% of the samples infected with at least one parasite taxa (Table 2).

Comparing the number of parasite taxa per sample for the infected samples, again Chunkar reserve had the lowest with an average of one taxa per sample. Kibber had an average of 1.27, Lari 1.82 and livestock samples from Kibber had 1.91 taxa per infected sample (Table 2). The percentage of samples infected by each of the 6 parasite species is given in Table 3. These results suggest that Chunkar reserve may provide a refuge from most parasite species.

The proportion of infected samples in AF, AM and young in the 3 locations is given in Table 4: Adult females in Kibber and Chunkar had a higher proportion (1 and 0.77, respectively) of parasite prevalence than AM (0.8 and 0.5) or young (0.9 and 0.43).

In our samples, parasite infection or prevalence and fGCM concentrations were not correlated, suggesting that these constitute independent measures of health for the blue sheep populations we studied.

Table 2. Details of sample collection for parasite analysis, and parasite prevalence for the different populations

Location	Human and livestock presence	Number of samples	Number of Parasite species found	Percentage of samples infected	Mean number of parasite species per infected sample
Kibber	Human + Large + Small livestock	33	6	87.80%	1.27
Chunkar	No humans or livestock	18	1	50.00%	1
Lari	Human + Large livestock	26	5	65.40%	1.82
Kibber livestock		27	5	81.50%	1.91

Table 3. Percentage of samples infected with the parasites (prevalence) in the four populations

Location	Ascaris	Trichuris	Strongyloides	Trichostrongyloides	Moniezia	Coccidia
Kibber	48.48%	0.00%	6.06%	3.03%	3.03%	51.51%
Chunkar	50.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Lari	61.54%	3.85%	15.38%	7.69%	0.00%	30.77%
Kibber livestock	44.44%	3.70%	14.81%	25.93%	22.22%	44.44%

Table 4. Proportions of samples infected among age-sex classes of blue sheep at the three locations

Location	Proportion of AF samples infected	Proportion of AM samples infected	Proportion of Young samples infected
Kibber	1	0.8	0.9
Chunkar	0.77	0.5	0.43
Lari	0.4	0.57	0.4

4. Discussion

This is the first study in the Indian trans-Himalayas on stress and parasite prevalence in blue sheep. We found that wild blue sheep had higher cortisol levels and parasite loads in areas grazed by domestic livestock. Moreover, females had higher cortisol levels and parasite prevalence than males.

Fecal sampling provides a convenient, non-invasive method to study the physiological responses of animals to stressors[26.27]. Many studies showed that anthropogenic disturbances increase stress levels in black grouse[11], owls[14], wolves and elk[9,13], coyotes[12], tigers[28], Capercaillie[10], etc. A few studies have shown increased stress levels due to cattle grazing in wood mice[16] and due to pastoralism in spotted hyenas in Masai Mara[29] and in tigers in Sariska, India[15]. Hence, this is the first study to examine stress response to livestock grazing in wild ungulates.

Previous studies have recorded that herbivores responded with elevated cortisol levels to resource scarcity, for instance in winter in red deer[30] and in the dry season in African elephants[31]. Similar to our findings, many studies have documented higher cortisol levels in females than males[27]. As we sampled in August-September, which is neither the mating season of blue sheep nor when females are pregnant, the differences between males and females in our study could either be due to female lactation (when they are more prone to stressors), or reflect baseline difference in sensitivity to stressors between the sexes as previously reported in other animals[15].

The increase of fGCM concentrations in Kibber and Tashigang is expected to be due to decrease of resource abundance in livestock-grazed areas. However, the samples from Lari had lower fGCM levels than samples from the Reserves which have even lower grazing intensities. We can speculate that the reasons for this pattern in Lari is the lowest altitude we sampled, and hence blue sheep may suffer less from physiologically adapting to the altitude at this location[32]. However, blue sheep are native to this region and are expected to be already adapted to the cold climate and high altitude. Lari is unique among the places we sampled in having apple cultivation, and it may be possible that this enriches blue sheep nutrition in this location (they are often seen feeding on the apple orchards, and get chased away when spotted by the villagers). It is possible that lower predator densities also contribute to the decreased fGCM levels[33,34]. Future studies are needed to test these considerations.

In general, human presence in the valley could cause increased fGCM levels through many factors. Villagers tend to chase away blue sheep from the crop areas. Vehicular traffic causes noise that may affect blue sheep. Human presence has also led to a high population of stray dogs, who act as predators for the blue sheep. The physical presence of humans and livestock may also act as stressors. Females may be more susceptible to all these factors due to the higher nutritional requirements of pregnancy and lactation. These stressors may also make the animals more prone to parasite infections, although we did not find any correlation between fGCM levels and parasite prevalence in our study.

Pathogen infections are of increasing concern to conservation studies[35]. Many studies have documented parasite transmission from domestic to wild animals[36], especially among ungulates[37,38], and have suggested limiting contact between domestic and wild mammals[39,40]. Many studies have shown increased parasite prevalence in human-dominated areas[20,40]. Previous studies have also documented significant population decline due to pathogen infections[37,41,42]. In a similar landscape to our study, it has been reported that overgrazing in the Tibetan plateau leads to greater transmission of *Echinococcus multilocularis* through increasing the population of small mammals[43]. Our study adds to this body of literature, finding that the protected Reserve at Chunkar offers a refuge from intestinal worms and protozoa.

All the parasites recorded in blue sheep in this study are also common parasites of goat, sheep and humans[44]. This result suggests that parasites might be transmitted from domestic animals to blue sheep, as most of the water bodies are shared between cattle, wildlife and people. Pastures are also shared between domestic livestock and wild herbivores, and there is open defecation by people. All these facilitate transmission of intestinal parasites between species[20]. Parasites found in our study, including strongyloides, trichostrongyloides and coccidia, can be quite harmful to the animals. Severe infection could lead to anemia, cause overall damage to the system, and affect reproduction and juvenile survival following weaning[45]. The higher percentage of young ones observed in the Reserve areas in this study may be the result of healthier populations in these areas, where we recorded lower parasite infection and prevalence. Lower parasite infections may also contribute to the better population performance recorded in ungrazed areas compared to grazed areas in previous studies on blue sheep in Spiti[7,8].

Our study suggests that presence of humans and livestock have significant effects on parasite prevalence and fGCM concentrations in blue sheep. Management recommendations include better management of wild population and resources, control of stray dog population by sterilization or culling, creation of exclusive protected areas for blue sheep, and control of grazing by domestic livestock in blue sheep protected areas. Further, periodic deworming of all domestic animals and people should also be carried out in the buffer area of blue sheep protected areas. These measures would certainly help in long term survival of blue sheep population in Spiti valley. In turn, this increases the wild prey base for the endangered snow leopards, possibly leading to decreased attacks on domestic livestock[2]. Hence, these measures may lead to both increase in the population of snow leopards and decrease in human-snow leopard conflict in Spiti.

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6. References

1. Mishra C. High altitude survival: Conflicts between pastoralism and wildlife in the Trans-Himalaya (Doctoral dissertation, Wageningen University, The Netherlands); 2001.
2. Nature Conservation Foundation. Management Plan for the Upper Spiti Landscape Including the Kibber Wildlife Sanctuary. Mysore, India; 2015.
3. Mishra C, Van Wieren SE, Heitkönig IM, Prins HH. (2002) A theoretical analysis of competitive exclusion in a Trans-Himalayan large-herbivore assemblage. Animal Conservation Forum (Cambridge University Press). 2002; 5(3):251-58. https://doi.org/10.1017/S1367943002002305
4. Handa O. Tabo Monastery and Buddhism in the Trans-Himalaya: Thousand years of existence of the Tabo Chos-Khor. Indus Publishing;1994.
5. Mishra C, Prins HH, Van Wieren SE. Overstocking in the Trans-Himalayan rangelands of India. Environmental Conservation. 2001; 28:279-83. https://doi.org/10.1017/S0376892901000297
6. Bagchi S, Mishra C, Bhatnagar YV. Conflicts between traditional pastoralism and conservation of Himalayan ibex (Capra sibirica) in the Trans-Himalayan mountains. Animal Conservation. 2004; 7(2):121-28. https://doi.org/10.1017/S1367943003001148
7. Suryawanshi KR, Bhatnagar YV, Mishra C. Why should a grazer browse? Livestock impact on winter resource use by bharal Pseudois nayaur. Oecologia. 2010; 162(2):453-62. https://doi.org/10.1007/s00442-009-1467-x PMid:19784849
8. Mishra C, Van Wieren SE, Ketner P, *et al*. Competition between domestic livestock and wild bharal Pseudois nayaur in the Indian Trans-Himalaya. Journal of Applied Ecology. 2004; 41:344-54. https://doi.org/10.1111/j.0021-8901.2004.00885.x
9. Creel S, Fox JE, Hardy A, *et al*. Snow mobile activity and glucocorticoid stress responses in wolves and elk. Conservation Biology. 2002; 16:809-14. https://doi.org/10.1046/j.1523-1739.2002.00554.x
10. Thiel D, Jenni-Eiermann S, Braunisch V, *et al.* Ski tourism affects habitat use and evokes a physiological stress response in capercaillie Tetrao urogallus: A new methodological approach. Journal of Applied Ecology. 2008; 45(3):845-53. https://doi.org/10.1111/j.1365-2664.2008.01465.x
11. Arlettaz R, Patthey P, Baltic M*, et al.* Spreading free-riding snow sports represent a novel serious threat for wildlife. Proceedings of the Royal Society of London B: Biological Sciences. 2007; 274:1219-24. https://doi.org/10.1098/rspb.2006.0434 PMid:17341459 PMCid:PMC2189568
12. Schell CJ, Young JK, Lonsdorf EV, Santymire RM. Anthropogenic and physiologically induced stress responses in captive coyotes. Journal of Mammalogy. 2013; 94(5):1131-40. https://doi.org/10.1644/13-MAMM-A-001.1
13. Millspaugh JJ, Woods RJ, Hunt KE, *et al.* Fecal glucocorticoid assays and the physiological stress response in elk. Wildlife Society Bulletin. 2001; 29(3):899-907.
14. Wasser SK, Bevis K, King G, Hanson E. Noninvasive physiological measures of disturbance in the northern spotted owl. Conservation Biology. 1997; 11(4):1019-22. https://doi.org/10.1046/j.1523-1739.1997.96240.x
15. Bhattacharjee S, Kumar V, Chandrasekhar M, Malviya M, *et al.* Glucocorticoid stress responses of reintroduced tigers in relation to anthropogenic disturbance in Sariska Tiger Reserve in India. PloS one. 2015; 10(6):e0127626. https://doi.org/10.1371/journal.pone.0127626 PMid:26061171 PMCid:PMC4465644
16. Navarro-Castilla A, Mata C, Ruiz-Capillas P, *et al.* Are motorways potential stressors of roadside wood mice (Apodemus sylvaticus) populations? PloS one. 2014; 9(3):e91942. https://doi.org/10.1371/journal.pone.0091942 PMid:24637740 PMCid:PMC3956862
17. Navarro-Castilla Á, Díaz M, Barja I. Does ungulate disturbance mediate behavioural and physiological stress responses in Algerian mice (Mus spretus)? A wild exclosure experiment. Hystrix. The Italian Journal of Mammalogy. 2017.
18. Gillespie TR. Noninvasive assessment of gastrointestinal parasite infections in free-ranging primates. International Journal of Primatology. 2006; 27:1129. https://doi.org/10.1007/s10764-006-9064-x
19. Sloss MW, Kemp RL, Zajac AM. Fecal examination: Dogs and cats. Veterinary clinical parasitology. 6th ed. Ames: Iowa State University Press; 1994.
20. Hussain S, Ram MS, Kumar A, *et al.* Human presence increases parasitic load in endangered lion-tailed macaques (Macaca silenus) in its fragmented rainforest habitats in southern India. PLoS One. 2013; 8(5):e63685. https://doi.org/10.1371/journal.pone.0063685 PMid:23717465 PMCid:PMC3661510
21. Umapathy G, Kumar V, Kabra, M, Shivaji S. Detection of pregnancy and fertility status in big cats using an enzyme immunoassay based on 5α-pregnan-3α-ol-20-one. General and Comparative Endocrinology. 2013; 180:33-38. https://doi.org/10.1016/j.ygcen.2012.10.009 PMid:23142266
22. Mithileshwari C, Srivastava T, Kumar V, *et al.* Non-invasive assessment of fecal progestagens and pregnancy detection in Himalayan musk deer (Moschus chrysogaster). Theriogenology. 2016; 85(2):216-23. https://doi.org/10.1016/j.theriogenology.2015.09.009 PMid:26454526

23. Kumar V, Reddy VP, Kokkiligadda A, *et al.* Non-invasive assessment of reproductive status and stress in captive Asian elephants in three south Indian zoos. General and Comparative Endocrinology. 2014; 201:37-44. https://doi.org/10.1016/j.ygcen.2014.03.024 PMid:24698789
24. Budithi NRB, Kumar V, Yalla SK, *et al.* Non-invasive monitoring of reproductive and stress hormones in the endangered red panda (Ailurus fulgens fulgens). Animal Reproduction Science. 2016; 172:173-81. https://doi.org/10.1016/j.anireprosci.2016.07.016 PMid:27481551
25. Umapathy G, Deepak V, Kumar V, *et al.* Endocrine profiling of endangered tropical chelonians using noninvasive fecal steroid analyses. Chelonian Conservation and Biology. 2015; 14(1):108-115. https://doi.org/10.2744/ccab-14-01-108-115.1
26. Keay JM, Singh J, Gaunt MC, Kaur T. Fecal glucocorticoids and their metabolites as indicators of stress in various mammalian species: A literature review. Journal of Zoo and Wildlife Medicine. 2006; 37:234-44. https://doi.org/10.1638/05-050.1 PMid:17319120
27. Touma C, Palme R. Measuring fecal glucocorticoid metabolites in mammals and birds: the importance of validation. Annals of the New York Academy of Sciences. 2005; 1046(1):54-74. https://doi.org/10.1196/annals.1343.006 PMid:16055843
28. Tyagi A, Kumar V, Kittur S, *et al.* Physiological stress responses of tigers due to anthropogenic disturbance especially tourism in two central Indian tiger reserves. Conservation Physiology. 2019; 7(1):coz045. https://doi.org/10.1093/conphys/coz045 PMid:31321036 PMCid: PMC6626984
29. Van Meter PE, French JA, Dloniak SM, *et al.* Fecal glucocorticoids reflect socio-ecological and anthropogenic stressors in the lives of wild spotted hyenas. Hormones and Behavior. 2009; 55(2):329-337. https://doi.org/10.1016/j.yhbeh.2008.11.001 PMid:19056392 PMCid:PMC2987620
30. Huber S, Palme R, Arnold W. Effects of season, sex, and sample collection on concentrations of fecal cortisol metabolites in red deer (Cervus elaphus). General and Comparative Endocrinology. 2003; 130:48-54. https://doi.org/10.1016/S0016-6480(02)00535-X
31. Foley CAH, Papageorge S, Wasser SK. Noninvasive stress and reproductive measures of social and ecological pressures in free-ranging African elephants. Conservation Biology. 2001; 15:1134-42. https://doi.org/10.1046/j.1523-1739.2001.0150041134.x
32. Beehner JC, McCann C. Seasonal and altitudinal effects on glucocorticoid metabolites in a wild primate (Theropithecus gelada). Physiology and Behaviour. 2008; 20:95(3):508-14. https://doi.org/10.1016/j.physbeh.2008.07.022 PMid:18706919
33. Sheriff MJ, Krebs CJ, Boonstra R. The sensitive hare: Sublethal effects of predator stress on reproduction in snowshoe hares. Journal of Animal Ecology. 2009; 78(6):1249-58. https://doi.org/10.1111/j.1365-2656.2009.01552.x PMid:19426257
34. Scheuerlein A, Van't Hof T, Gwinner E. Predators as stressors? Physiological and reproductive consequences of predation risk in tropical stonechats (Saxicola torquata axillaris). Proceedings of the Royal Society of London B: Biological Sciences. 2001; 268(1476):1575-82. https://doi.org/10.1098/rspb.2001.1691 PMid:11487404 PMCid: PMC1088780
35. Lafferty KD, Gerber LR. Good medicine for conservation biology: The intersection of epidemiology and conservation theory. Conservation Biology. 2002; 16:593-604. https://doi.org/10.1046/j.1523-1739.2002.00446.x
36. Gortázar C, Ferroglio E, Höfle U, *et al.* Diseases shared between wildlife and livestock: a European perspective. European Journal of Wildlife Research. 2007; 53:241. https://doi.org/10.1007/s10344-007-0098-y
37. Vander Waal KL, Atwill ER, Isbell LA, McCowan B. Quantifying microbe transmission networks for wild and domestic ungulates in Kenya. Biological Conservation. 2014; 169:136-146. https://doi.org/10.1016/j.biocon.2013.11.008
38. Martin C, Pastoret PP, Brochier B, *et al.* A survey of the transmission of infectious diseases/infections between wild and domestic ungulates in Europe. Veterinary Research. 2011; 42:70. https://doi.org/10.1186/1297-9716-42-70 PMid:21635726 PMCid:PMC3152899
39. Pedersen AB, Jones KE, Nunn CL, Altizer S. Infectious diseases and extinction risk in wild mammals. Conservation Biology. 2007; 21(5):1269-1279. https://doi.org/10.1111/j.1523-1739.2007.00776.x PMid:17883492 PMCid:PMC7202242
40. Gortazar C, Diez-Delgado I, Barasona JA, *et al.* The wild side of disease control at the wildlife-livestock-human interface: a review. Frontiers in Veterinary Science. 2015; 1:27. https://doi.org/10.3389/fvets.2014.00027 PMid:26664926 PMCid:PMC4668863
41. Leendertz FH, Pauli G, Maetz-Rensing K, et al. Pathogens as drivers of population declines: the importance of systematic monitoring in great apes and other threatened mammals. Biological Conservation. 2006; 131:325-37. https://doi.org/10.1016/j.biocon.2006.05.002
42. Daszak P, Cunningham AA, Hyatt AD. Infectious disease and amphibian population declines. Diversity and Distributions. 2003; 9:141-150. https://doi.org/10.1046/j.1472-4642.2003.00016.x
43. Wang Q, Xiao YF, Vuitton DA, et al. Impact of overgrazing on the transmission of Echinococcus multilocularis in Tibetan pastoral communities of Sichuan Province, China. Chinese Medical Journal-Beijing. 2007; 120(3):237.https://doi.org/10.1097/00029330-200702010-00013
44. Sanyal PK. Gastrointestinal parasites and small ruminant production in India. In:ACIAR Proceedings; 1996. p. 109-112.
45. Roberts LS, Janovy J, Schmidt JD. Foundations of Parasitology, New York: McGraw Hill; 2009.