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Original Article

Investigation on Parasites and some Causes of Mortality in Captive Punjab urial (*Ovis vignei punjabiensis*), Pakistan

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Abstract

Background: The present study was conducted in Jul 2019 and Jan 2020 in two wildlife parks of the Nowshera district, Khyber Pakhtunkhwa, Pakistan, where the endangered Punjab urial (*Ovis vignei punjabiensis*) is successfully bred in captivity. We determined diversity of internal and external parasites that take advantage of the situation of congestion, resulting in massive mortalities of wild animals in captivity.

Methods: Internal parasites of living urial were determined by direct wet smear and flotation methods, while dead urial was necropsied for any pertaining observation.

Results: All examined fecal samples were found infected with gastrointestinal parasites, which had significant difference in the total abundance in winter and summer. *S. papillosus* and *H. contortus*, and a single protozoan, *Eimeria* spp. were the dominant parasites in fecal samples. Ticks collected from urial enclosures and dead animals were of single species *H. anatolicum*. *Theileria* spp. was observed in blood, while hydatid cysts were found in lungs and liver of necropsied urial.

Conclusion: The study indicates that internal parasites such as *Haemonchus contortus* and *Strongyloides papillosus*, while external parasites as *Hyalomma anatolicum* ticks played major role in the population decline. Strict veterinary control of infectious diseases, provision of hygienic and supplementary diet, and proper maintenance of urial population are necessary measures for the control of mortalities.



Introduction

Zoos and wildlife parks serve different purposes to wildlife, the first being provision of shelter to handicapped animals that cannot survive in nature on their own. Zoos also provide shelter to exotic confiscated animals that cannot be released in local environment due to the probability of competition with or biological invasion on the local fauna. Many zoos around the world provide study workplace to scientists in reproductive biology for the reintroduction of animals, especially of that facing extinction threat (1, 2). Human activity affects natural habitats and wildlife behavior; hence, for the education and awareness of public, raising wild animals in captivity has become both a social and scientific necessity. However, zoos face an undeniable problem of infectious diseases, due to too much congestion of different types of wild animals in such limited space. Parasites could be host-specific for infecting a single host type or could be generalists for infecting a wide variety of hosts (3).

Urial is a species of wild sheep inhabiting India, Pakistan, Afghanistan, eastern Iran, and Central Asian countries. Urial (*Ovis vignei*) and Asiatic mouflon (*Ovis orientalis*) are distinct species, because urial has 58 chromosomes, while Asiatic mouflon, which inhabits western Iran and Turkey, has 54 chromosomes (4). Urial of Pakistan are single species, and have less genetic difference (5). Three subspecies are generally recognized to occur in Pakistan, viz Ladakh urial (*Ovis vignei vignei*), Afghan urial (*Ovis vignei blanfordi*) and the Punjab urial (*Ovis vignei punjabiensis*) (6, 7). The validity of these subspecies still warrants the need of enormous research. Nonetheless, the Punjab urial (*O. v. punjabiensis*) occupies parts of central Pakistan, declared endangered (8).

In Pakistan, research on diseases of captive wild sheep and goats is in deficit. At present, most of the old yet important biological publications on fauna of the country have not been

digitized and remained mostly inaccessible to readers. However, case reports on urial parasites could be found. However, Awan et al.'s (9) suggestions largely set the trend of research on parasites of urial at the population level. Later, blood parasites (10) and other causes of mortality (11) were studied in Punjab urial. Punjab urial of Kalabagh and Afghan urial of Torghar have many parasites common with domestic sheep and goats (12, 13).

The present study was conducted to investigate ecto and endoparasites as well causes of mortalities in captive Punjab urial at two wildlife parks of Khyber Pakhtunkwa Province, Pakistan.

Materials and Methods

Study areas and animals

The current study was conducted in Jul 2019 and Jan 2020 at two wildlife parks in Nowshera district of Pakistan. Both these parks are currently serving as promising breeding facilities for Punjab urial (1). Cherat Wildlife Park (CWP) covers an area of 26.97 km² established in 2006. Manglot Wildlife Park (MWP) covers an area of 7.12 km² established in 1990. The animals were occasionally drenched with anthelmintics. However, so far have never been vaccinated against viral or bacterial diseases. Urial is maintained in vast enclosures of the park. The enclosures were made near human settlement especially in CWP, without considering effect of human disturbance or transmissible diseases of livestock on the caged wildlife. Human, domestic animals and stray dogs pass outside, yet very close to the enclosures. So far these urial have not been reintroduced anywhere.

Ectoparasite sampling

During the current study physical capture or restrain the animals was not allowed. For sampling ticks from ground, we used two

methods, the visual searching method and the dragging method (9, 14). In each enclosure five, 60 meter long transects were designed. The visual searching method was based on searching the ground and tips of leaves along the transect, especially the grasses and shrubs. In the dragging method, we used a cotton sheet (2x1m), dragged it slowly and carefully over the transect, and checked it for ticks after every 10 m. Specimens were carefully collected to avoid damaging body parts and were preserved in glass bottles containing 70% ethanol. All the samples were identified under a stereo microscope up to species level by consulting the given descriptions (15).

Fecal analyses

Overall, 68 fecal samples were collected from living urial of both study sites, in summer and winter. Samples were collected in polythene bags and taken to Veterinary Research Institute, Peshawar. Fecal samples were left at room temperature for a few days for the emergence of third-stage larvae (L_3) from nematode eggs. All the samples were processed and examined by flotation in saturated salt solution. Eggs and larvae were identified consulting different diagnostic literature (15, 16). Eggs/larvae/oocysts per gram of feces (E.P.G., L.P.G., O.P.G.) were determined by McMaster technique.

Postmortem reports

Based on postmortem reports, we retrospectively analyzed mortality causes of captive Punjab urial at CWP (Dec 2008-Jan 2020) and at MWP (Dec 2012- Jan 2020). Overall, 52 urial died at CWP, and 7 at MWP. The animals were classified into three age groups, i.e., lambs (<1 yr old), yearlings (≥ 1.5 yr old) and adults (≥ 2 yr old) (17). Death causes were determined based on autopsy examinations. Some of the tissue samples, adult helminths and cysts were preserved in 70% ethanol. To study hemoprotozoa, blood was obtained from the heart, smeared thinly on slides, fixed

in absolute ethanol and treated with Giemsa staining.

Statistical analysis

Data sets were analyzed using the SPSS ver. 20 (IBM, Chicago, IL, USA). Descriptive statistics was used for investigating number of infected organisms and percentages. The Pearson's chi-square test was used to analyse the prevalence of all infected groups with total parasitic infestation. Variables were significant at $P < 0.05$.

Results

Gastrointestinal Parasites

All the studied fecal samples in both seasons were infected with one or more gastrointestinal parasites. Overall, eight nematodes species were identified, i.e., *Strongyloides papillosus*, *Haemonchus contortus*, *Marshallagia marshalli*, *Chabertia ovina*, *Ostertagia* spp., *Trichostrongylus* spp., *Trichuris* spp. and *Nematodirus* spp. Protozoa belonging to *Eimeria* genus were observed (Table 1). At both study sites, the gastrointestinal helminths were more common than the protozoa. *S. papillosus* (19.89%), *H. contortus* (19.3%), *Trichostrongylus* spp. (14.6%) and *Trichuris* spp. (7.6%) were the dominant parasites.

Seasons had significant influence on overall abundance of parasite E.P.G., L.P.G., and O.P.G. at both sites. *S. papillosus* had significantly high mean value of the total seasonal abundance of of E.P.G. and L.P.G. at both sites ($P=0.0001$). *H. contortus* had no significant difference on abundance of infection ($P > 0.05$) in both seasons. Whereas, *Eimeria* spp. had significant difference between two seasons in only CWP ($P=0.016$).

Ectoparasites

Overall, 78 unfed ticks specimens were collected in both seasons from CWP, whereas no specimen was collected from MWP. All these specimens belonged to *H. anatolicum*. Female ticks were more abundant ($n=53$) than male ticks ($n=25$).

Table 1: Percentage prevalence and mean intensity of parasites in Punjab urial of CWP and MWP

Variable	Summer		Over- all Sum- mer Preva- lence (%)	Winter		Overall Winter Preva- lence (%)
	CWP (n=23) Preva- lence (%)	MWP (n=11) Preva- lence (%)		CWP (n=23) Preva- lence (%)	MWP (n=11) Preva- lence (%)	
Overall helmin- thosis	100	-	100	-	100	100
<i>Strongyloides papillosus</i>	100	858.70 ± 45.6	100	1000 ± 108.7	100	-
<i>Haemonchus contortus</i>	30.4	326.1 ± 83.7	-	-	20.6	73.9
<i>Chabertia ovina</i>	13	287.5 ± 42.7	-	-	8.8	-
<i>Ostertagia</i> spp.	17.4	480.0 ± 107.9	-	-	11.8	-
<i>Trichostrongylus</i> spp.	26.1	930.0 ± 78.4	27.3	516.7 ± 72.6	26.5	30.4
<i>Trichuris</i> spp.	21.7	440.0 ± 87.2	36.4	662.5 ± 110.6	26.5	21.7
<i>Marshallagia marshalli</i>	-	-	45.5	340.0 ± 64.0	14.7	-
<i>Nematodirus</i> spp.	-	-	36.4	412.5 ± 108.7	11.8	-
<i>Eimeria</i> spp.	82.6	1260.5 ± 91.6	9.1	1500 ± 0.0	58.8	100
						750 ± 42.6

Postmortem changes

The postmortem results showed a significant difference between mortalities in different age groups in both sites. Lambs were the most susceptible age group showed high rates of mortalities (61%). We categorized postmortem findings under two heads, parasite-related and physiology-related observations with a highly significant difference ($\chi^2=58.9$; $P=0.003$) (Table 2). Four types of parasites were found in dead animals, i.e., tick infestation with *H. anatolicum* (22%), hydatid cysts in both lungs (3.45%) and liver (10.16%), *Theil-*

eria spp. (6.8%), and *H. contortus* stomach (3.4%). The physiological anomalies noted were weak musculature (18.6%), gastrointestinal infections (8.5%), haemorrhagic diarrhea (8.5%) and cardiac arrest (3.4%). Overall mortalities and parasites infections were higher in females than males (Fig.1). Miscellaneous causes of mortality constituted 5.1% in males, and 10.2% in females. It included heat stroke, predation, stillbirth, snakebite, and deaths occurring due to land sliding, or during physical capturing of animal for drenching, and natural age-related causes.

Table 2: The percentage of Punjab urial that died at CWP (2009-19) and MWP (2014-19)

Postmortem observations	CWP (%)			MWP (%)			Total
	Lamb	Yearling	Adult	Lamb	Yearling	Adult	
Parasitological observations:							
Ticks infestation	6 (10.2)	2 (3.4)	5 (8.5)	-	-	-	13 (22)
Hydatid cyst infection	6 (10.2)	1 (1.7)	1 (1.7)	-	-	-	8 (13.6)
<i>Theileria</i> infection	4 (6.8)	-	-	-	-	-	4 (6.8)
<i>Haemonchus</i> infection	2 (3.4)	-	-	-	-	-	2 (3.4)
Physiological observations							
Weak musculature	8 (13.6)	-	-	3 (5.1)	-	-	11 (18.6)
Gastrointestinal infections	2 (3.4)	1 (1.7)	2 (3.4)	-	-	-	5 (8.5)
Haemorrhagic diarrhea	2 (3.4)	1 (1.7)	-	1 (1.7)	1 (1.7)	-	5 (8.5)
Cardiac arrest	-	-	2 (3.4)	-	-	-	2 (3.4)
Miscellaneous	2 (3.4)	1 (1.7)	4 (6.8)	-	-	2 (3.4)	9 (15.3)
Total	32 (54.2)	6 (10.2)	14 (23.7)	4 (6.8)	1 (1.7)	2 (3.4)	59 (100)
	$\chi^2 = 37.8 (P=0.036)$			$\chi^2 = 22 (P=0.005)$			$\chi^2 = 58.9 (P=0.003)$

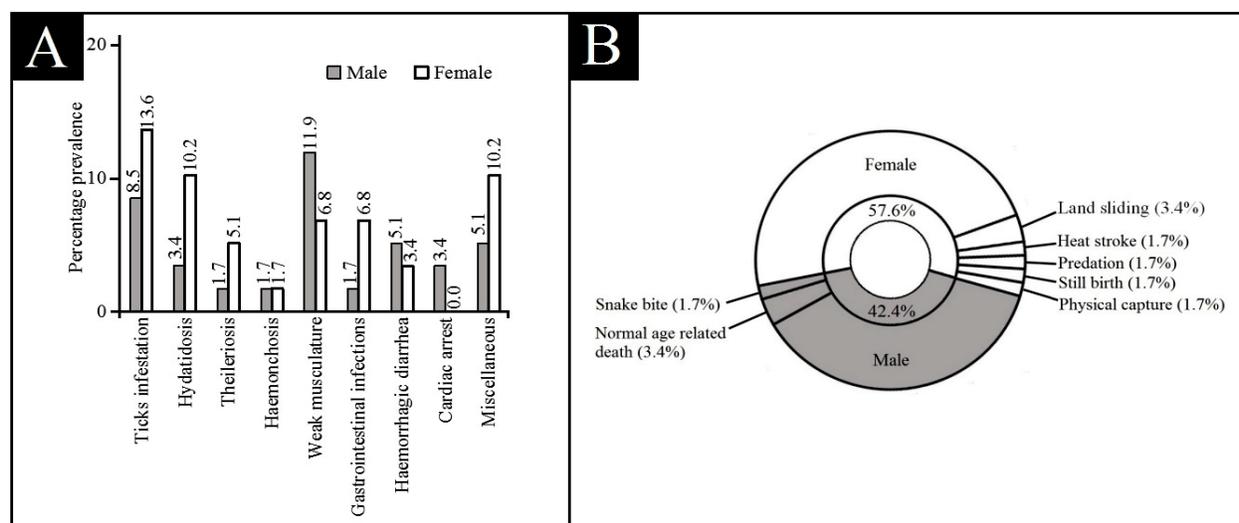


Fig. 1: Collective sex-wise data of urial mortalities in CWP and MWP. (A) Parasitological and physical anomalies were noted during postmortem. (B) Overall mortality percentage and miscellaneous causes of death

Discussion

The parasitic fauna recorded during the present study remarkably resembled other reports from nearby Peshawar city (18). Hence, the common parasites of small ruminants of this region in Pakistan are species of *Fasciola*, *Haemonchus*, *Trichuris*, *Trichostrongylus*, *Strongyloides*, *Ostertagia* and *Marshallagia*, which depend on moisture for their existence. The present study

was a thorough investigation of infection with parasites and causes of mortality in captive Punjab urial. Nowshera district through winter and summer, where the two wildlife parks of our study are located, is a humid (0.017-0.069 mm) and riverine region (19). Necropsy of dead animals is necessary, as it shows parasites that cannot be otherwise observed in coprological studies. The present study is the first to report hydatid cysts in urial of Pakistan. This

canine-borne metacestode of *Echinococcus granulosus* tapeworm is widely prevalent in domestic sheep and goats of Pakistan (20), and also reported in Himalayan ibex (*Capra ibex sibirica*) of India and Asiatic mouflon (*Ovis orientalis*) of Iran and Turkey (13).

During present research, *Eimeria* spp. was recorded. Species of *Eimeria* are commonly seen associated with captive and free-living wild sheep of Iran (21-23). In Pakistan, seven species of *Eimeria* were found in Afghan urial, with prevalence of *E. ovinoidalis*, *E. bakuensis* and *E. parva* to be comparatively higher than others were (13). *Eimeria* is host-specific, which means sheep *Eimeria* cannot infect goats or other animals (24). Yet, urial, mouflon and domestic sheep belong to the same genus and are closely related to each other. Hence, they share many parasites, including *Eimeria*.

During the present research, *Theileria* spp. and its vector *Hyalomma anatolicum* ticks were recorded in Punjab urial, both being more prevalent in females, than in male urial. Both these parasites are widely prevalent in small ruminants of Pakistan, especially in the areas near Nowshera district (25-28). Anatolian wild sheep of Turkey were found infected with *Rhipicephalus turanicus*, *R. bursa* and *Hy. excruciatum* (29, 30), the latter two ticks also found associated with *Theileria ovis* (30). In Iran, 16 species of ixodid ticks were reported from wild sheep (mouflon, urial and their hybrids) and wild goats (bezoar), including *Hy. anatolicum excruciatum* from Trans-Caspian urial (31). The earliest study in Pakistan regarding ticks of wild animals led to collection of *Ha. sulcata*, but not *Hy. anatolicum* from Punjab urial in Jhelum district (32). Recently, PCR studies documented presence of *T. ovis* in Punjab urial (10). Small ruminants of Pakistan are parasitized by many more blood protozoa and ticks genera than discussed in this work (25-28), and all of them have strong potential to infect urial.

Mortality in captive wild mammals is always associated with overcrowding of animals in limited space (1, 2, 11). Parasites proliferate so well, especially when host animals are available at little distance. Mortality is sure when the parasite has a dramatic effect on body condition. In our study, we suspect four parasites to have caused mortalities in urial, i.e., *S. papillosus*, *H. contortus*, *Hy. anatolicum* and *Theileria* spp., though other parasites are not exempted. The nematode *S. papillosus* causes dramatic weight loss (33), and in heavy infections, it leads to cardiac arrhythmias and sudden death (34, 35). The other nematode *H. contortus* causes blood loss, yet we did not perform blood count of animals. Heavy ticks' infestation cause paralysis of limbs in the early stage and mortality in a later stage. Tick paralysis and respiratory failures contributed to the 46% mortalities of captive Anatolian wild sheep (30). In Punjab urial of Lahore zoo, tick infestation, tick paralysis and theileriosis were seen in 20% of the mortalities cases (11). We strongly hold ticks to be the forerunner in causing mortalities in urial here, because 78 ticks were collected from CWP where 53 urial had died, while in MWP only 7 urial had died, with no ticks infestation. Besides parasitological causes, deficiency in the nutritional values also contributes to weak musculature (11). In addition, the captive populations are more prone to inbreeding depression, resulting in weak progeny and ultimately higher mortalities. No shifting of breeding animals from both the facilities in the current study was one of the many limiting factors (1, 2).

Conclusion

Clumping of animals in limited space increases mortality, as was the case with captive Punjab urial in our study. The animals were infected with two protozoa, eight nematodes, one metacestode and one arthropod parasite. Together, these parasites, especially *Haemonchus*, *Strongyloides*, *Hyalomma* and *Theileria* con-

tributed particularly in driving the urial population to a dramatic fall. While free-living urial may live well with these parasites, the captive animals succumb due to genetic, nutritional, and behavioral stresses associated with captivity. The links of communicable disease between wildlife and domestic animals need to be disconnected by eliminating wildlife-livestock interaction, by provision of veterinary care, and by treating and vaccinating for other potential diseases. Translocation of breeding pairs, release of surplus animals in their natural habitat or other enclosures, and recruiting adults from wild habitats are necessary for preserving genetic diversity of the endangered wildlife in captivity.

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Conflict of interest

The authors declared that there is no conflict of interest.

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