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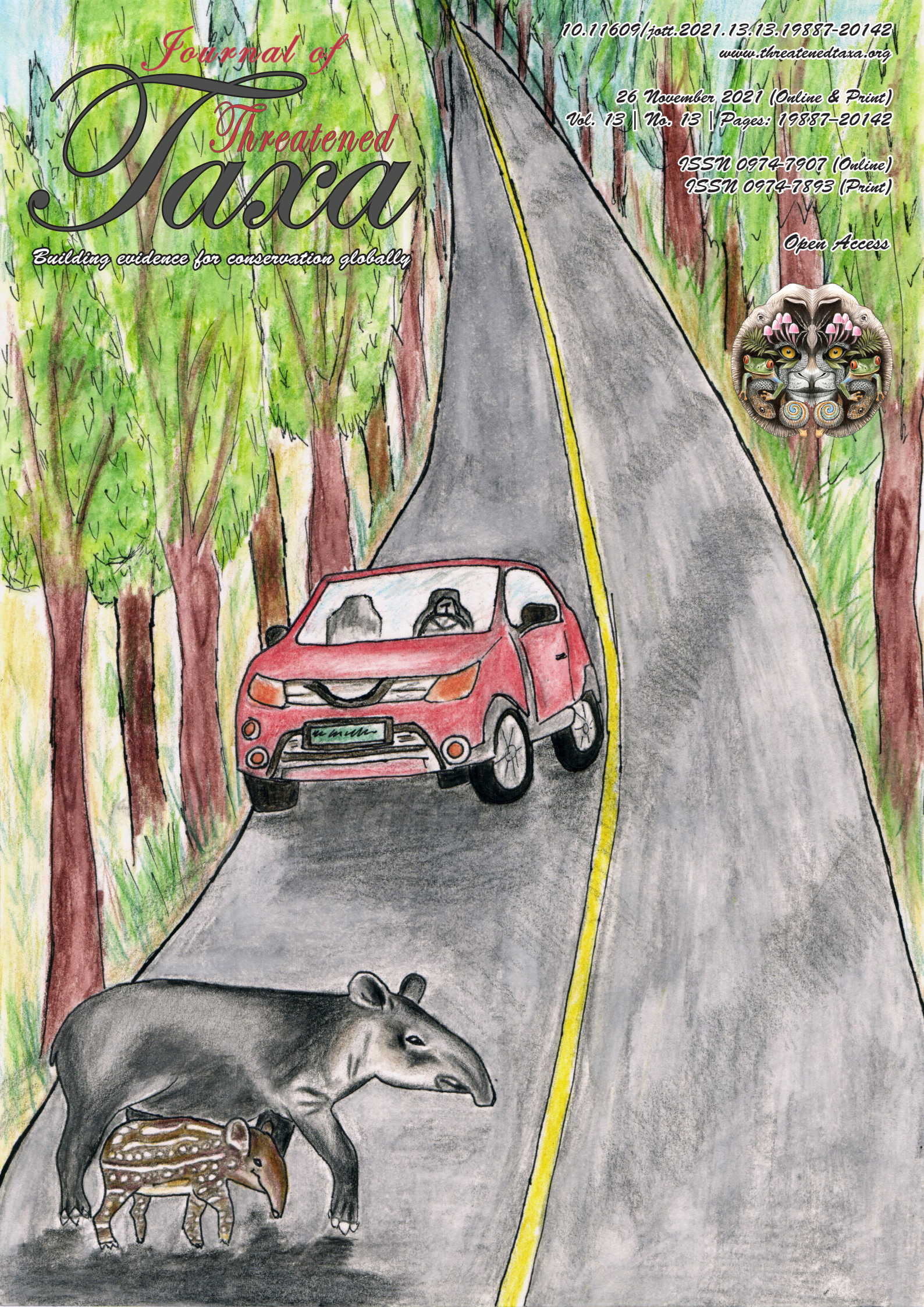

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continued on the back inside cover

Caption: Lowland Tapir *Tapirus terrestris* (Medium—watercolours on watercolour paper) © Aakanksha Komanduri.

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NOTE

Study on incidence and pathology of gastrointestinal parasitic infections in Nilgai *Boselaphus tragocamelus* in Hisar, Haryana, India

Maneesh Sharma [1], B.L. Jangir [2], D. Lather [3], G.A. Chandratre [4], V. Nehra [5], K.K. Jakhar [6] & G. Narang [7]

[1] Department of Veterinary Clinical Complex, [2–7] Department of Veterinary Pathology,
College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS) Hisar, Haryana 125004, India.
[1] drmaneeshvet@gmail.com (corresponding author), [2] drbabu.jangir@gmail.com, [3] deepikalather@yahoo.co.in,
[4] drgaurichandratre@gmail.com, [5] drvikasnehra@gmail.com, [6] kk_hau@rediffmail.com, [7] gulshan.narang@gmail.com

India has a diverse population of domestic and wildlife animals that are either kept in captivity or in free range. Among them, Blue Bull, commonly called Nilgai in India, is one of the most commonly observed wild animals in agriculture dominated landscapes in central and northern India (Meena et al. 2014). The main threat for the existence of this species is the loss of their habitat due to human population growth, encroachment of forests and emergence of wildlife diseases. Several studies of wild life diseases especially parasitic diseases have been carried out in wild animals including nilgai species (Banerjee et al. 2005) and a number of helminths, arthropods and protozoan parasites were reported. In wild conditions, animals have some natural resistance against parasitic diseases and there is a state of equilibrium between the parasite and the host and it seldom led to harmful infection unless stressed (Gaur et al. 1979; Mir et al. 2016). In India, studies to assess helminth fauna of wild animals under captivity were carried out in a systematic way but the literature on the parasitic infections of free ranging wild animals is very scanty because of the difficulty in collection of fresh samples from these free ranging wild animals. The samples collected during postmortem cases may contribute in a better understanding of parasitic load as well as pathology caused by them to formulate different strategies in the control of different parasitic diseases.

Study area: The present study was conducted in the Department of Veterinary Pathology, Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS), Hisar. The Hisar district of Haryana is located between 29.151 latitude, 75.721 longitude and 215.5m altitude. The climate of Hisar region is continental and it lies at the outer margins of the monsoon region, 1,600 km away from the ocean. The average maximum and minimum temperature during the month of March is 31.5°C and 16.4°C. The average annual rainfall in the district is 455 mm(Central Ground Water Board 2017).

Collection and processing of the samples: A total of 20 carcasses of Nilgai were brought to the Department

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Funding: None.

Competing interests: The authors declare no conflicts of interest with respect to the research, authorship, and/or publication of this present article.

Ethical approval: In the present article, samples are taken from the dead carcasses of Nilgai brought to Veterinary Pathology for complete postmortem examination. Hence, it does not need any ethical approval and is not under consideration elsewhere and none of the paper's contents have been previously published.

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of Veterinary Pathology for necropsy by Wild Life Department, Hisar, from Hisar district and surrounding areas. Intestinal contents and faecal samples were collected in sterile polythene bags, properly sealed, labelled and processed for detailed routine parasitological analysis for the presence of parasitic eggs/oocysts by direct smear method and standard flotation technique using a saturated salt solution and sedimentation method.

Pathological studies: The detailed gross pathological examination was carried out and lesions were recorded. For histopathology, the representative tissue samples of intestine were collected in 10% neutral buffered formalin. After proper fixation, tissue samples were processed for histopathological examination. The tissues were properly trimmed, washed in running tap water over night, then dehydrated in ascending grades of ethanol, cleared in xylene and then embedded in paraffin wax. Sections of 4–5 µm thickness were cut using a semi-automatic rotary microtome. Thereafter, the sections were stained with haematoxylin and eosin (H&E) as per standard procedure (Luna 1968).

Results: During necropsy, the external examination of most of the carcasses showed varying degrees of traumatic injuries (9), external wounds and haemorrhages (5) along with putrefactive changes (6). Grossly, intestines revealed reddish discoloration, thickened mucosa and the presence of catarrhal exudate (Image 1). All 20 faecal samples were screened for the presence of gastrointestinal helminthsand protozoan infections. Overall the incidence of parasitic gastrointestinal tract infection was 40% (8/20). All eight cases revealed the presence of coccidian oocysts (Image 2), along with mixed infection with one or more types of helminthic eggs in two samples. All coccidian infections were found to be caused by *Eimeria* species by using sporulation technique. Among helminthic infections, *Strongyle*, *Moneizia* and *Trichuris* spp. were observed. Faecal samples showed unsporulated oocysts of *Eimeria* spp., thin double walled, smooth colourless eggs of *Strongyle* spp. containing blastomere (Image 2), eggs of *Moneizia* spp. containing a distinct pyriform apparatus (Image 3) and yellowish-brown, barrel shaped thick walled eggs of *Trichuris* spp. (Image 4) possessing a pair of polar plugs at both ends and egg mass identified by morphological characteristics. The results of faecal sample examination are presented in Table 1 and 2. Microscopic examination of intestine revealed congested mucosal and submucosal blood vessels, fusion of villi and desquamation of mucosal epithelium in focal areas (Image 5). Intestinal mucosa also revealed moderate infiltration of mononuclear cells mainly lymphocytes in the lamina propria along with different developmental stages of coccidian oocysts (Image 6).

Table 1.Incidence of parasitic infection in Blue Bull / Nilgai (N=20 faecal samples).

Oocysts/ Eggs of parasites observed	Number of samples
Coccidian oocysts (*Eimeria* spp.)	06
Mixed infection (*Eimeria*spp. + *Moneizia* spp. +*Strongyle* spp.)	01
Mixed infection (*Eimeria*spp. + *Strongyle* spp. + *Trichuris* spp.)	01
No parasitic infection	12
Total	**20**

Table 2. Semi quantitative load of parasitic eggs/oocysts in Nilgai.

	Sample	Parasitic load			
		***Eimeria* spp.**	***Moneizia* spp.**	***Strongyle* spp.**	***Trichuris* spp.**
1	N1	-	-	-	-
2	N2	-	-	-	-
3	N3	-	-	-	-
4	N4	-	-	-	-
5	N5	-	-	-	-
6	N6	++	++	+++	-
7	N7	-	-	-	-
8	N8	+++	-	++	+
9	N9	-	-	-	-
10	N10	-	-	-	-
11	N11	++	-	-	-
12	N12	++	-	-	-
13	N13	++	-	-	-
14	N14	+++	-	-	-
15	N15	++	-	-	-
16	N16	+	-	-	-
17	N17	-	-	-	-
18	N18	-	-	-	-
19	N19	-	-	-	-
20	N20	-	-	-	-

-—No egg/oocyst | +—Mild load | ++—Moderate load | +++—Heavy load.

Discussion: Wild animals are important reservoirs and amplifiers of emerging human and domestic animal pathogens including parasitic infections. In addition to their well-recognized zoonoses of public health significance, wildlife has gained considerable attention in recent years. Parasitic infections are quite common in wild ruminants across India (Banerjee et al. 2005). These

Image 1. Gross examination of intestine revealed congestion, haemorrhages, thickened mucosa and presence of catarrhal exudates.

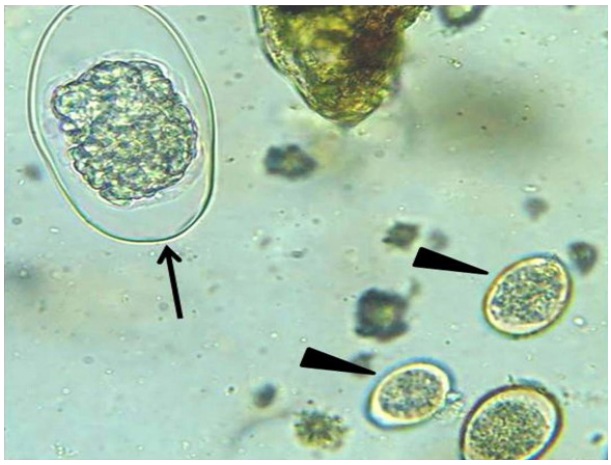

Image 2. Faecal sample showing thin double walled, smooth colourless barrel shaped egg of Strongyle spp. containing blastomere (arrow) and unsporulated oocysts of Eimeriaspp. (arrow heads). X 400.

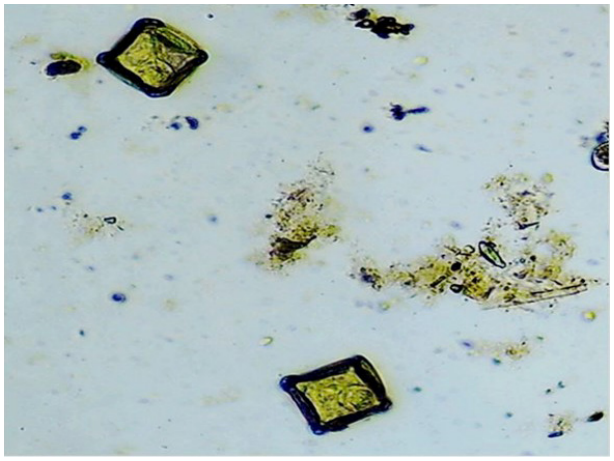

Image 3. Faecal sample showing square eggs of Moneizia spp. containing a distinct pyriform apparatus. X 400.

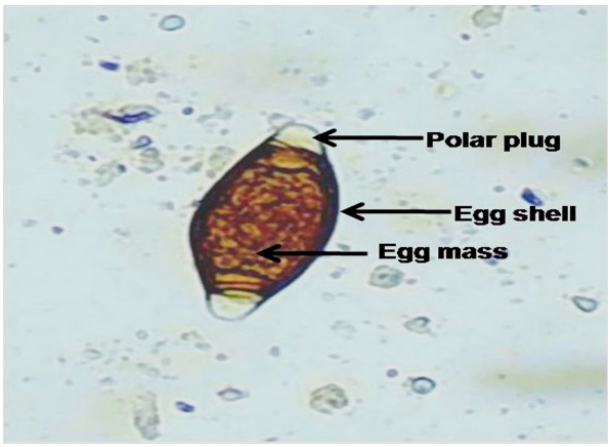

Image 4. Faecal sample showing barrel shaped thick walled eggs of Trichuris spp. possessing a pair of polar plugs at both ends and egg mass. X 400

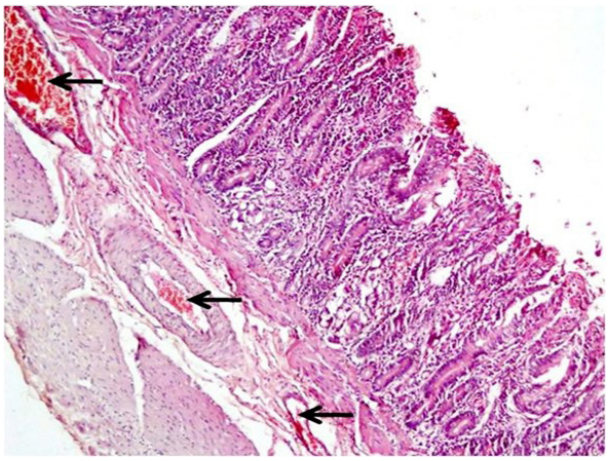

Image 5. Section of intestine showing congestion (arrows), fused villi, desquamated mucosal epithelium in focal areas and infiltration of mononuclear cells mainly lymphocytes. H&E X 100

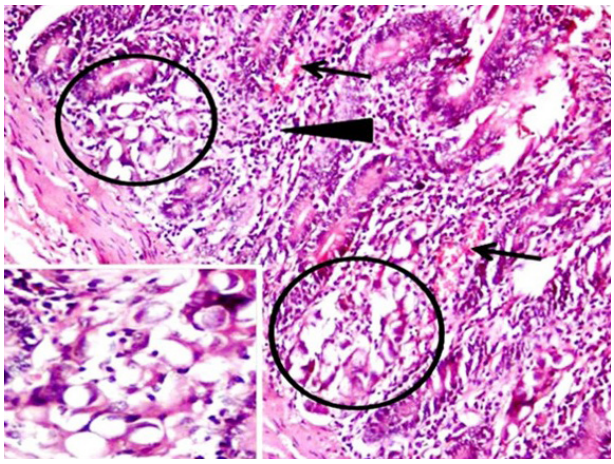

Image 6. Section of intestine showing congestion (arrow) of intestinal mucosa revealed moderate infiltration of mononuclear cells mainly lymphocytes (arrow) and different stages of developmental stages of coccidian oocysts (encircled). H&E X 400.

animals may acquire large quantums of infection while grazing that are mostly subclinical. However, sometimes the infections turn fatal for the infected animal. In the present study, most of the carcasses of Nilgai examined showed varying degrees of traumatic injuries, external wounds and haemorrhages and putrefactive changes. The main reason for the death of the animals is due to accidental injuries from vehicles on the roads and the use of barbed wires by farmers for fencing to protect agricultural land. However, this can be minimized by use of alternative methods of fencing to protect crop damage (Meena et al. 2014). The extent of injuries varied from bruises, lacerations and single to multiple fractures cases. Similar finding of varying degrees of more or less traumatic injuries caused by bullets/gunshots, infighting, automobile/train accidents, jumping/falling leading to haemorrhagic shock was also reported by Sharma et al. (2014).

As regard to parasitic infections, the morphological characteristics of the eggs of *Strongyle* spp., *Moneizia* spp., *Trichuris* spp., and coccidian oocysts was confirmed as per Soulsby (1982). Various studies of single and mixed parasitic infections in wild animals has also been reported by earlier researchers (Abhishek et al. 2011; Jaiswal et al. 2014). In Uttaranchal, India, 41.6% of 161 faecal samples from Nilgai were positive for single or mixed infections of *Amphistomes*, *Strongyles*, *Trichuris*, *Fasciola*, and *coccidians* (Banerjee et al. 2005). Endoparasitic fauna in wild animals and consequent detection of infection in these wild animals suggests close interactions with domestic animals (Holsback et al. 2013). Sharing of the same pasture land and water bodies like ponds by wild and domestic animals might be a potential source of infection for domestic animals. Histopathologically, intestines revealed circulatory disturbances such as congestion, enteritis and different developmental stages particularly in coccidian infected cases. Similar findings were also observed in Nilgai by other researchers (Sharma et al. 2012). In the present study, different types of mixed parasitic infections in free ranging nilgai with gross and microscopic changes in the intestinal tracts indicate that the increase in parasitic load might be due to the secondary infestation by opportunistic parasites due to decreased immune response because of stressful environmental conditions or injuries. Further, more detailed studies will be required as wild herbivores not only come into close contact with different domestic animals, but share the same pasture for grazing that might cause potential threat of interspecies transmission. This will also be of great importance for species conservation.

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