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Prevalence of intestinal helminth parasites in *Macaca mulatta* and their unmanaged consequences in Nepal

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Abstract. A total of 202 fecal samples from *Macaca mulatta* (Rato Bandar) from Pashupati and Nilbarahi areas were microscopically examined to determine the prevalence and gastrointestinal species helminth parasites. Fecal smears were prepared from fresh fecal samples and microscopically examined. About 3 g of the dropping was also preserved separately in clean, properly labeled vials containing 10% formalin. Out of total samples (202), 124 (61.38%) samples were found positive for one or mixed infection of more than one helminths, and 78 (38.61%) samples were hostile to any helminths. Egg of 17 helminth species (15 nematodes, 1 trematode, and 1 Acanthacephala) was identified. Out of the total samples (202), the nematodes were the most prevalent with a class-wise prevalence percentage (95.16%), and trematode was the least prevalent with the Acanthocephala (5.64%). Infection with nematode *Strongyloides fulleborni* was found to be the most prevalent with 51.61%, whereas prevalence with nematode *Toxocara canis* and *Cooperia species* were found to be the least 0.80%. The prevalence percentage of intestinal helminth parasites from Rhesus monkeys was 64.70% on Pashupati and 58% in Nihbarahi.

Keywords*: Gastrointestinal parasite, samples, parasitic prevalence, rhesus monkey, Nepal*

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1. Introduction

Man gives pride to himself and the apes, monkeys, and lemurs because they are higher mammals. These animals have many of the same structural characters as men. Some of them resemble him more in one feature, some in another. They are grouped with the man in a typical natural order, the Primates, the first the highest of animals according to their brain's superior development. At the other end of the scale is man, who stands alone and unapproachable among all mental endowment creatures. The immeasurable superiority of the human intellect marks the line of cleavage between man and animal.

Three species of macaques have been reported in Nepal. The Rhesus monkey (*Macaca mulatta),* Assamese monkey (*Macaca assamensis),* and Langur monkey (*Presbytis entellus*) (Chalise and Ghimire, 1998). The rhesus monkeys, *Macaca mulatta,* are the most common of all these species in the world. They are distributed in South and East Asia, i.e., Afghanistan, Bangladesh, Burma, China, Hong-Kong, India, Nepal, Srilanka, and Thailand. In Nepal, they are usually found in jungles of religious spots like Pashupatinath temple, Swayambhunath temple, Dakshinkali, Ram temple, and Nilbarahi and natural forests Gokarna and Charkoshi Jhadi (terai). They are even found in Kathmandu Valley's localities, such as Tripureshwor, Thapathali, Sankhu, and Pulchowki Dada (Chalise and Ghimere, 1998; Yadav, 2004)**.** *Macaca mulatta* is commonly called Rato Bandar in Nepali, Rhesus Monkey in English, Lal Bandar in Hindi, Makkad in Marathi, Baojha Hindko, Mankad in Oriya, Kathi in Telugu, and Bandar in Urdu.

The rhesus macaque *(Macaca mullata*) has a relatively short tail, uniformly well brown hair with much bright color, more reddish -hind parts. The bare face of rhesus is light pink, flesh-colored, and reddish. The ears of rhesus are pointed and protruding. Rhesus bearded special pads on limbs. Forelimbs and hind-limbs are of equal size in rhesus. They are primarily arboreal in their habitat (Napier and Napier, 1985). The weight of male rhesus monkeys ranges from 5.6-10.9 kg, and in females, the weight ranges

from 3.0-10.7 kg. The head and body length in males reach up to 48.5-63.5 cm, and in females, it ranges from 47.0-53.1 cm. The tail length in males ranges from 20.3-30.5 cm, while in females, it ranges from 19.0-28.5 cm (Roonwal and Mohnot, 1977). Rhesus Macaque occurs low lying flat lands to the foot of Himalaya up to 4000m (Chalise et al., 2005).

The helminths (Helminth = worm) are worm-like parasites. These are multicellular and bilaterally symmetrical elongated, flat, or round bodies. Helminths are broadly classified into Flatworms or Platyhelminthes (Platy = flat), including flukes and tapeworms and Roundworms or Nematodes (Nemato = thread), include nematodes. Since helminth parasites are the causative agent of a terrible and list of debilitating, deforming, and killing diseases of primates, the studies in these regards are critical from an epidemiological perspective. Furthermore, some helminth infection, particularly intestinal parasites, is one of the major causes of gross health problems. Rhesus monkeys are always used as experimental animals by the medico-biological institute. So this species is targeted for the study purpose. Being higher primates, many organ systems are similar to men. Studies on Rhesus monkeys concerning intestinal helminth parasitic diseases have not been carried out yet in Nepal. This is the first attempt to study them.

2. Materials and Methods

2.1 Study area and sample collection

The study was conducted in both Pashupati (Kathmandu) and Nilbarahi (Bhaktapur) of Nepal. Pashupati is an ancient religious place lying at a distance of 5 km northeast of Kathmandu city. This area lies 1302.79 m above sea level. Physically, it is situated between 85°20'57" to 85°21'04" and 27°42'20" to 27°42'26" east longitude and north latitude (Pashupati Vikas Kosh). Most of the Pashupati areas are under the influence of the human community. Nilbarahi is also an ancient religious place in the Bhaktapur district. It lies 1335 m above sea level. Physically, it is situated 85°23'30" to 85°24'30" and 27°40'30" to 27°42'30" east longitude and north latitude. Stool samples were collected from June to December. Samples were preserved 5% formalin or 2, 5 potassium dichromate according to the availability.

2.2. Examination of stool samples

Examining stools was first carried by naked eyes to know whether there were adult worms [*A lumbricodes*, hookworm, T.T, E *vermicularis* various intestinal flukes or a part of it (the segment of tapeworms)]. After that, a microscopic examination of stool was carried out to identify helminth eggs and larvae. This was done by the wet mount of fresh stool, preserved specimen, or fecal concentrates.

2.3. Direct smear

The following process was carried out: saline direct, wet mount, wet mount, and wet iodine mount. For the identification of helminth and species present. After examination of the direction for further investigation, used the concentration method.

2.4. Concentration method

After direct routine examination, the concentration technique was performed because this technique increases the ability to detect helminth eggs and larvae by decreasing the number of background materials in the preparation and an actual concentration of organisms.

Floatation techniques. In the floatation technique, the suspending fluid (sodium chloride or zinc sulfate) has higher specific gravity than the parasitic forms, which, therefore, rise to the surface. All the helminthic eggs float in such a solution except the following: -unfertilized eggs of *A. lumbricoides*, eggs of *Taenia solium* and *Taenia saginata,* eggs of an intestinal fluke. The strongholds' larvae do not float in the salt solution.

They saturated the salt floatation technique. About 3 g of stool sample was taken. That stool was kept on the sieve and kept on the porcelain basin and ground. About 42 ml of water was then added and again ground and filter. The stool solution's filtrate was mixed, and about 15 ml was kept on the plastic tube. The tube was centrifuged at 1000 rpm to 5 min. Then without disturbing the sediment, the above solution was removed slowly. The tube was kept on the stand, and sodium chloride solution was added to the upper level so that the upper meniscus of water could be seen. Now cello tape was kept on it and was centrifuged at 1000 rpm till 5 min. Cello tape was taken out and kept on the slide, and was observed under the microscope. From this process,

eggs of Nematode, Cestode, and Coccidian can be detected. For the detection of lungworm, the half saturated salt solution is used.

Concentration zinc sulfate solution technique. This technique is used to detect tapeworms. Sodium chloride solution was removed from the tube, which has been prepared from the above-prepared procedure. The stool, which has been sediment at the bottom of the tube and covered by transparent cello tape, can contact the solution. It was then centrifuged at 1000 rpm for 5 min. Cello tape was taken out and kept on the slide, and was observed under the microscope. By this process, eggs of *Fasciola* and *Paramphistome* can be observed.

Sedimentation technique. Suspension of stool was prepared as a floatation method. The suspension was filtered through the sieve in the beaker. The beaker was filled with water. The solution was sediment for 10-20 min, and the above water was thrown away. Again the beaker was filled with water. Again allow the solution to sediment for 10-20 min. Then the above water was removed, and sediment was kept on a Petri dish. One drop of methylene blue was added to it and was observed under a microscope.

2.5. Identification of the eggs

The identification of the eggs was confirmed by comparing the structure, color, and size of the eggs. These were also compared with that of the chart in CVL.

2.6. Statistical analysis

The data were analyzed by the SPSS V.18 (SPSS Inc., Chicago, USA). The data are significant for the Tests were considered statistically significant at a *p*-value of ≤ 0.01.

3. Results and discussion

3.1. Intestinal helminth parasites

Intestinal parasites are cosmopolitan in distribution—all animals, whether humans, domestic animals, or wild animals, bear different parasites. Many kinds of research have been carried out regarding the intestinal parasites of humans, because of always eager to know about our health for the interested wild animal and domestic for veterinary. The study intestinal helminth parasite of monkeys who belong to the wildlife population has been carried out. The research so far has not been adequate, especially in the Nepalese context. Despite Nepal's research work's inadequacy, it is considered quite justified to compare it with the statistics of international research work.

Toxocara Canis. *Toxocara canis* completes its life cycle in a single host. The adults usually inhabit the intestinal tract of the dog, wild jackal, and fox. The adult worms are not found in man. The females are relatively longer than the males and measure 1-6 cm in length and 4-6 mm in breadth. The egg is round with the characteristic pitted shelf and measures 75 by 85 mm. The eggs do not contain an embryo at the time of their excretion in the faces. *Toxocara canis* is in dogs from Kathmandu (Sing, 1970).

Toxascaris leonine. *Toxascaris leonine* occurs in the small intestine of dog, cat, fox, wild Felidae, and Canidae in most parts of the world. The males are up to 7 cm, and the females are up to 10 cm—the infective stage in the egg containing a second-stage larva. *Toxascaris leonina* in lepard (Sing, 1970).

***Ascaris lumbricoides*.** The adult male measures 15-30 cm in length and 3-4 mm in diameter, whereas females are relatively larger than the males. A female measurement was 20-40 cm in length and 2-6 mm in diameter. Both fertilized and unfertilized eggs are present in the faces. Fertilized eggs were oval to subspherical in shape and measure 45-70 μm in length and 35-50 μm in breadth. They are bile-stained and golden brown. Unfertilized eggs are brown thin-shelled ellipsoidal and measure 78-105 μm in length and 38-55 μm in breadth. These are the heaviest of all the helminthic eggs; hence they do not float in the saturated salt solution.

***Ostertagia* sp.** The adults are slender reddish-brown worms up to 1 cm long. The egg measures 74-90 μm in length and 40-45 μm in size. Acharya (1999) reported *Ostertagia* species in sheep and goats of IAAS livestock, central lab, Tripureshwor.

Haemonchus conturtus. Freshly acquired specimens are generally reddish due to the host's blood contained within. The adult worms are distinguished by a single curved lancet in the small buccal cavity's depth and by a pair of spine-like cervical papillae. Male (10-20 mm by 0.4 mm) is smaller than females (18-30 mm by 0.5 mm). The eggs are oval, thin-shelled, and hyaline,

elongated, and measure 75-90 μm by 40-50 μm. They are already segmented when deposited and resemble those of Trichostrongylus. In 1999, Joshi reported *Haemonchus conturtus* in sheep and goats from Karki district Pokhara.

***Cooperia* sp.** Adults are reddish when fresh male measures 4.5-0.4 mm in length whereas female measures 5.8-6.2 mm in length. In 1997, Joshi reported *Cooperia* sp. In goat sheep and buffalo from Kathmandu.

Ancylostoma duodenale. The worms are cylindrical, grayish-white, and slightly curved worms. The worm's anterior end is bent slightly, in the same direction of the body curve, and gives its name "hookworm." Males (8 mm in length) are smaller than females (12.5 mm in length). The eggs are oval (60 μm in length and 40 μm in breadth), thin-shelled, and non-bile stained. The eggs usually contain seven to eight blastomeric- a bright space present between the segmented ovum and the eggshell. Sharma and Tuladhar (1971) reported *Ancylostoma duode*nale in humans from Kathmandu.

***Dictyocaulus* sp.** The adults are slender thread-like worms, and females are 5.0-11.0 cm in length. Their location in the trachea and bronchi and their size are diagnostic. Eggs are 112-138 μm to 69-90 μm in size, ellipsoidal, and contain fully developed larva when laid, or first stage larva may pass in faces. In 1982, ADPCD reported *Dictyocaulus* sp. in goat and sheep from Kathmandu.

***Chabertia* sp.** The adult worms are stout and are white. Males are 13-14 mm in length, and females are 17-20 mm in length. The life cycle is direct. Worms are found attached to the colon's mucosa, congested, swollen, covered with mucus, and punctiform hemorrhages may be present. Eggs are laid in the morula stage, and it measures 90-105 μm in length and 50-55 μm in breadth. Joshi (1997) reported *Chabertia ovina* in goat and sheep from the western hills of Nepal.

***Oesophagostomum* sp.** The adult is tiny and is covered with a coticule- the male measures 8-10 mm in length and 0.35 mm in breadth. Females are 8.5-10 mm long and 0.35 mm thick. The eggs are usually indistinguishable from those of Ancylostoma and Necator. They measure 60-63 μm by 30-40 μm. In 1999, Acharya reported *Oesophagostomum* sp. in sheep and goat Central lab, Tripureshwor.

***Trichostrongylus* sp.** The male measures 4-5 mm in length, and the female measures 4-6.5 mm in length. Eggs are oval and bilaterally symmetrical. These are relatively larger (63-115 μm by 40 μm) than those of hookworm ova. The shell has a thin transparent outer chitinous layer and a thin inner layer. The embryonic mass is multi-segmented and varies from 16-32 in number. The space between the eggshell and embryonic mass is relatively conspicuous. Joshi (1997) reported *Trichostrongylus colubiformis* cattle and goat from the western hills of Nepal.

***Oxyuris* sp.** Mature females are giant white worms with pointed tails, which may reach 10 cm in length, whereas mature males are generally less than 1 m long. Eggs are asymmetrical, 80-90 μm in length and 40-45 μm in breadth, flattened one side with an operculum, often pugged with mucus at one pole, and passed in the faces in an advanced morula stage.

Strongyloides fulleborni. The adult female is hardly visible to the naked eye and measures 2.5 mm by 0.04 mm. The life cycle is completed in a single host. The life cycle is unique due to its potential for autoinfection and multiplication within the infected host. The eggs are more or less similar to that of *Trichuris* sp, but in comparison to Trichuris egg, the shell is almost colorless, the egg is barrel-shaped, with the asides nearly parallel. The egg's size is smaller than that of trichuris, i.e., 44-50 μm by 24-33 μm. The color pale brown but not bile stained. They had fine striations in the eggshell. Rabwin et al. (2003) reported *Strangles* in the horse from Kyanjingompa, Langtang.

Trichuris trichura. *Trichuris, trrichura*, also called the whipworm, gets its name from its characteristics whip-like shape. The length of the body ranges from 30-40 mm in males and 40-50 mm in females—the worm feed on epithelial cells and blood. The life cycle is direct, with no intermediate loss. Copulation occurs in the lumen of the host's intestine. Afterward, the female lays eliminated along with the faces. The eggs' development takes place in the moist soil, and the infective juveniles are formed in about 3 weeks. At each end, eggs have barrel-shaped with a colorless protruding mucous plug. These are yellowish-brown and double-shelled. The outer shell is bile-stained. These measures were 50-54 μm in length and 22-23 μm in breadth. These float in the saturated salt solution. The eggs contain an unsegmented ovum each when passed in the faces.

***Trichuris ovis*.** Males are 50-80 mm in length, and females are 35-70 mm in length. The eggs are 70-80 μm in length and 30-42 μm in breadth, unsegmented, brown in color, and barrel-shaped with a transparent plug at either pole. In 1988, Gupta reported *Trichuris trichura* in humans from Kirtipur. Joshi (1997) reported *Trichuris ovis* in goat and sheep from the western hills of Nepal.

***Dicrocoelium* sp.** The adult fluke is lancet-shaped, is transparent, and measures 5-15 mm in length and 1.5-2.5 mm in breadth. The eggs always hatch inside the snail to release the miracidia. The latter undergo two generations of sporocysts to

develop into cercariae. Redia stage is absent. The cercaria is spinous. Eggs are operculated, yellowish-brown, thick-shelled, and measure 38-42 μm, and 22-30 μm. The eggs are mature, containing the mirecidia when freshly laid. Gauri et al. (2007) reported *Dicrocoelium lanceatum* in buffaloes from Satungal Kathmandu.

Prosthenorchis elegans. Adults are 2-5 cm long, and the proboscis is globular with five to seven rows of hooks. The intermediate host is cockroaches (*Blatella germanica*), which may be shared in primate colonies. This species has not yet been reported from Nepal in any host. The eggs measure 65-81 μm by 2-53 μm, containing the thick outer and thin inner shells enclosing the embryo (acanthor). Eggs are oval. *Prosthenorchis elegans* is reported for the first time in Nepal. All the genus and species of the intestinal helminth parasites observed in the Rhesus monkey are reported here for the first time from Nepal. Whereas, *Prosthenorchis elegans* is reported for the first time from Nepal. In 1982, ADPCD reported *Capillaria* sp in poultry from Kathmandu.

3.2. Prevalence rate

General. Out of 202 fecal samples examined, 124 samples were positive for one or more than one helminth parasite, and the remaining 78 were negative. Hence, the positive percentage was 61.38%, and the negative percentage was 38.61%. In the present study, 202 fecal samples were examined from the two study sites. Out of the 202 fecal samples were examined, positive 124 samples for one or more than one helminth parasites and the remaining 78 were negative. Hence the positive percentage was 61.38, and the negative percentage was 38.61. Thus, the percentage of the damaging helminth parasite was much lower than that of the positive one. This is quite natural since (1) the monkeys eat food and water, most of which are contaminated, (2) and not dewormed. Ponnudurai et al. (2003) found that 51% of the monkeys in Tamil Nadu tested positive for the parasitic helminth infections, while the present study undertaken is 61.38%. This difference in the prevalence rate could be attributed to the difference in climate and topography.

Class-wise prevalence rate. Out of 124 positive samples, 111 samples (89.51%) were positive for nematodes 7 samples (5.64%) were positive for Acanthocephala, and 6 samples (4.83%) were positive for trematodes. Statistically, a significant difference was found in class-wise parasitic infection. ($\chi 2=371.19$, $P\leq 0.05$). Some identified and unidentified protozoans were also detected during fecal samples examination for monkey's intestinal helminth parasites.

Table 1. Prevalence percentage from the Pashupati area of Kathmandu district and Nilbarahi area of Bhaktapur district.

Study Area	Total samples examined	Positive samples for helminths	
		No.	Percentage (%)
Pashupati	102	66	64.70
Nilbarahi	100	58	58.00
Total	202		

The comparative prevalence rate of helminth parasites in Pashupati and Nilbarahi areas. The prevalence rate of helminth in *Macaca mulatto* Pashupati is found to be more than that in Nilbarahi. Statistically, a significant difference was found in area-wise parasitic infection ($\chi 2=17.77$, $P\leq 0.05$) (Table 1). The percentage of 64% is in Pashupati and 58% in Nilbarahi.

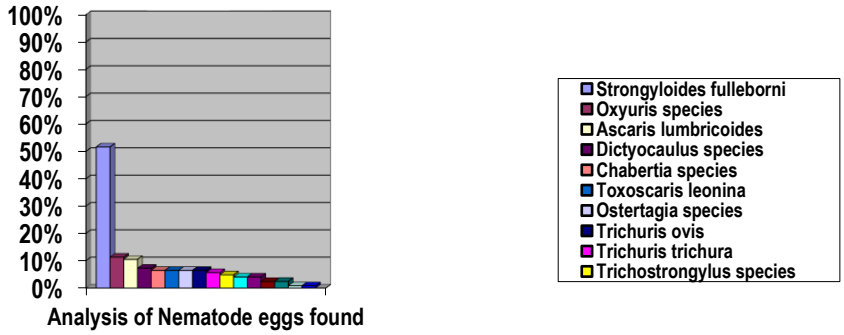

Figure 1. Nematode eggs found.

Prevalence percentage of specific nematode parasites. Out of the 124 positive samples, 111 were favorable to the different Nematodes (Figure 1). The Nematodes with the most significant prevalence percentage was *Strongyloides fulleborni* with 51.61% prevalence percentage. The least prevalence rate was of *Toxocara Canis* and *Cooperia* species with a prevalence percentage of 0.80.

The prevalence rate of specific Helminthes. Out of 202 stool samples, Nematode (*Stongyloides fulleborni*) was found to be the most prevalent with a prevalence rate of 51.61%, while the nematodes (*Cooperia* species and *Toxocara canis*) were found to be the least prevalent with a prevalence rate of 0.80% (Figure 2). The prevalence rate of other species of nematodes were as follows *Oxyuris species*-11.29%, *Ascaris lumbricoides*-10.48%, *Capillaria species*-10.48%, *Dictyocaulus species*-7.25%, *Chabertia species*-6.45%, *Toxascaris leonine*-6.45 %, *Ostertagia species* -6.45%, *Trichurs ovis* -6.00%, *Trichuris trichura* -5.64%, *Trichostrongylus species* -4.83%, *Capillaria* -4.48%, *Oesophagostamum species* -4.03 %, *Ancylostoma duodenale* -2.41%, *Haemonchus conturtus* -2.14 %. *Cooperia species* -0.80 %, *Toxocara canis* -0.80 % comparatively.

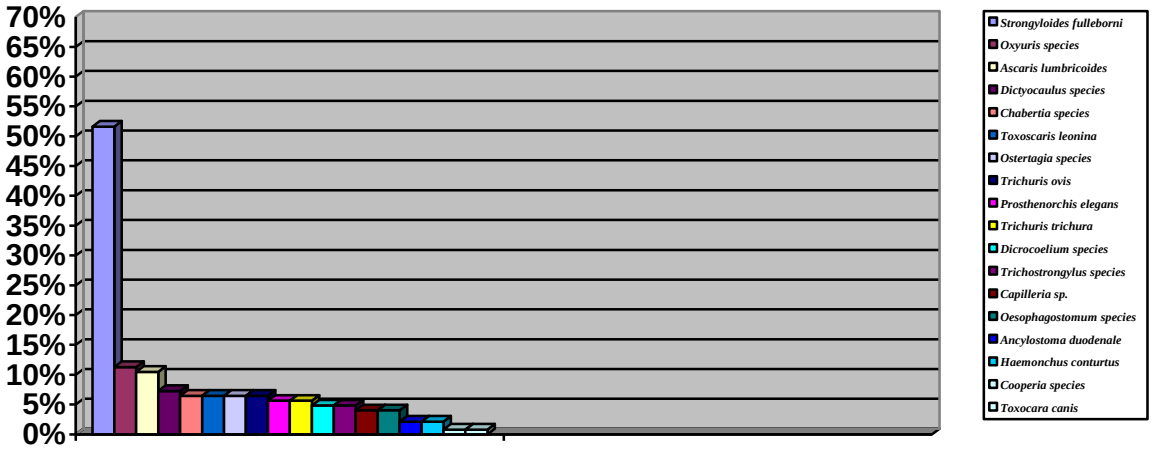

Figure. 2. Prevalence percentage of the specific helminths.

The prevalence rate of zoonotically infective helminthes of *Macaca mulatta*. Out of total samples (202), 108 samples were positive to zoonotically infective helminths, 16 were adverse to zoonotically infective helminths, and 78 were hostile to any helminths (Table 2). Adhikari (2017) investigated the multiple, triple, double, and single-species 2.15%, 6.45%, 29.03% and, 36.55% similar to that of the Pokherel and Maharjan (2014) recorded 1.17%, 4.7%, 17.65%, 43.53%.

Table 2. The prevalence rate of zoonotically infective helminth parasites.

Total no. of Stool Samples examined	Negative to any Helminth egg	Positive to the helminth egg					
		Helminth (Overall)		Zoonotically infective helminth		Zoonotically non-infective helminth	
		No.	%	No.	%	No.	%
202	78	124	61.38	108	87.09	16	12.90

4. Conclusion

We conclude that the present study of the prevalence of intestine helminth parasites between the monkeys of Pasupatinath and Nilbarahi. Zoonoses are those disease and infections which are transmissible between man and animals. Since monkeys belong to the primate order and many monkeys' organ systems are similar to humans, they harbor mansu zoonotically infective helminth. In the present study 1 *Trematode Dicrocoelium sp*. and 10 nematodes, namely *Strongyloides fulleborni, Oesophagostamum sp, Capallaria Trichostrongylus, Ascaris lumbricoides, A. duodenale, Haemoinchus conturtus, Cooperia sp. Ostergis sp.* and *Toxocara canis,* which are already known to be zoonotically infective, were found. *Prosthenorchis elegans* is reported for the first time in Nepal.

Conflicts of interest. The authors declare that there is no conflict of interest.

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