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Research Article

Analysis of Intestinal Parasites in Deer Family under Captivity in Lahore Zoo

Iftikhar Ali[1]*, Syed Rooh-u-llah[1], Haroon Akbar[1], Samra Khan[1] and Mahnoor Pervez[2]

[1]School of Zoology, Minhaj University, Lahore, Pakistan; [2]Department of Zoology, Faculty of Science and Technology, Lahore College for Women University, Lahore

Abstract | Present study was conducted on 5 species of deer family (Hog deer, Spotted deer, Fallow deer, Red deer and Sambar deer) kept at Lahore zoo. The Fecal samples of each deer family were collected, analyzed and identified using standard techniques. Six gastrointestinal parasites i.e. *Buxtonella*, *Blantidium coli*, *Trichostrongylus sp*., *Moniezia*, *Toxocora* and algal cysts were found in the fecal samples of all captive deer families. Worm load indicated that the rate of parasitic infestations in autumn and winter seasons was not uniform in all species of captive deer. The *Blantidium coli* and *Buxtonella* load increased (p>0.05) in Hog deer, Spotted deer, and Sambar deer but decreased in Fallow deer and Red deer. Eggs per Gram (EPG) of *Trichostrongylus sp*. exhibited an increasing trend (p>0.05) in Hog deer but significantly decreased (p>0.05) in all other deer families indicating that a decrease in temperature negatively impacted parasitic growth. *Toxocora sp.* increased during winter in Hog deer and Spotted deer only. *Moniezia sp.* exhibited an increase (p>0.05) with respect to a decrease in temperature specifically in Fallow, Red and Sambar deers. Algal cyst were not observed in Hog, Spotted and Fallow deers but a sizeable quantity of said cysts were seen in Red and Sambar deers both in autumn and spring seasons. However there was almost 50% decrease in algal cysts in Fallow and Sambar deers in winter seasons, implicating the effects of temperature and humidity. Over all the findings of current study indicated a definite infestation of different parasites in all species of deer family kept at Lahore zoo. It was further concluded that numbers and species of parasites prevalent in deer families varied in relation to the seasonal temperature and humidity.

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***Correspondence** | Iftikhar Ali, School of Zoology, Minhaj University, Lahore, Pakistan
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Introduction

The scientific name of deer species is *Cervidae*. Deer are sociable creatures. Usually, they gather in little groups for mating, foraging, and defence. Depending on the availability of food and the population make-up, truly huge herds

can emerge. Deer family belongs to the species of ruminants, they favour nutritious food that is simpler to digest because antler growth requires a lot of energy and nutrition (Uresk and Dietz, 2018).

The Hog deer (*Axis porcinus*) is the least studied wildlife species in Pakistan. It is a member of the *Cervidae* family and the Order *Artiodactyla*. It is a little deer with little, delicate legs and a big, massive body that is a dark olive hue in color. Because most of them are hunted and raised for their meat, hides, and antlers, hog deer are economically significant. The skin of these animals is used to make gloves, coats, and footwear. Knives and button handles are made from the antlers (Kanungo *et al*., 2010). Fallow deer (*Damadama*) is a kind of ruminant mammal that belongs to the *Cervidae* family. It is thought to have originated in Turkey, the Italian peninsula, the Balkan Peninsula, and the European island of Rhodes. It was originally indigenous to prehistoric Europe and was dispersed throughout a broader area of the continent (Lemoine and Svenning, 2022). For farmed Red deer (*Cervus elaphus*), lungworm and gastrointestinal nematode parasites are serious health problems. Chronic gastrointestinal nematode infection and periodic, frequently pathogenic lungworm breakouts in young deer have led many farmers to heavily relying on anthelmintic treatments. The creation of more comprehensive and long-lasting control measures depends on a better understanding of the epidemiology of the parasites afflicting farmed deer, particularly the origins and seasonality of pasture infection on the farm (Chambers *et al*., 2022). The IUCN Red List classifies the Sambar (*Rusa unicolor*), a sizable deer that is native to Southeast Asia and the Indian subcontinent, as a threatened species. Populations have significantly decreased as a result of intense hunting, local rebellion, and industrial habitat exploitation. The *Javan rusa* known as the "Sunda sambar" and the Philippine deer known as the "Philippine Sambar" are occasionally referred to as "Sambars" (Singh *et al*., 2020).

Parasites that develop without any intermediate hosts significantly affect the gastro-intestinal tract of definitive hosts, making helminthic infection a serious health concern in captive and wild animals (Panayotova-Pencheva, 2013). The host's health is adversely affected by parasites, and deer life is greatly threaten by multiple parasites especially Nematodes and Flukes that may leave hundreds of eggs and cyst in the abdomen and intestinal tract which can be seen in feces with different examination methods (Rauque *et al*., 2011). Grazing ruminant animals are almost always infected with gastrointestinal parasites, even if fecal egg shedding is lower than the detectable level, it is widely considered that the stock is infected. The most popular method for determining gastrointestinal parasite infection in animals is microscopic examination which can be used to identify the eggs, oocysts, and larvae that intestinal parasites shed in the feces of their hosts (Verocai *et al*., 2020).

The aim of the study was to determine the frequency and seasonal variations of major parasites *Blantidium coli*, *Buxtonella*, *Trichostrongylus sp*., *Toxocora sp*. and algal cysts in Hog deer, Spotted deer, Fallow deer, Red deer and Sambar deer in Lahore zoo. The study was conducted to improve our understanding of potential sources of pasture contamination.

2. Materials and Methods

2.1. Study area

The current study was conducted at Lahore Zoo. All deer families kept in Lahore zoo were selected for research work, including all male and female animals.

Figure 1: Map of Lahore Zoo

2.2. Experimental design

The fecal samples of each animal from all deer species were collected immediately after defecation from the ground and then placed into polyethene bags. Feces were randomly and manually collected, similar to pool sampling. The bags were then tied securely and numerically numbered. The fecal samples were collected at 6:00 am in the morning after every 2 weeks for each animal during the whole experimental period. They were freshly analyzed in laboratory or chilled at 4°C for further testing. In order to evaluate the fecal samples, the Direct, Flotation, and McMaster examination methods were used.

2.3. Parasitic examination

2.3.1. Direct method

About 1 g of fresh feces was taken from each of the 5 fecal bags constituting a total of 5 grams fecal palette which was calculated using an electronic scale. Then, by using a pestle and mortar, the feces pallet was crushed into tiny granules and preserved in a 200 ml beaker, 2.5 ml of distilled water was added to the beaker comprising the fecal sample for further softening, and it was then pass through the sieve to separate out the debris. The filtrate was gathered for additional examination.

2.3.2. Flotation method

After centrifugation of the solution (to remove all the debris), a 35 ml solution of sodium chloride was added in the filtrate. Due to their low specific gravity, parasitic eggs started to float after two to three minutes. The eggs were extracted and examined microscopically.

2.3.3. McMaster method

For McMaster examination of eggs, the filtrate, after the centrifugal separation was put on a McMaster Slide chamber using a dropper. Refined samples were examined under a 10x objective lens compound microscope. With the aid of the identification keys, the eggs were recognized. Some of the eggs were also cultured for further accurate identification of eggs or cysts.

3. Results

3.1. EPG of gastrointestinal parasites

3.1.1. Hog deer

Total six types of parasites namely *Blantidium coli, Buxtonella, Trichostrongylus sp, Toxocora sp., Moniezia* and algal cysts were observed in collected fecal samples (Figure 2). The Eggs per Gram (EPG) of *Blantidium coli, Buxtonella, Trichostrongylus sp, Toxocora sp., Moniezia* was 3600, 1400, 1050, 560, 50, 0 respectively (Table 1).

Blantidium coli was more persistent in hog deer than *Buxtonella*. Moderate presence of the parasite *Trichostrongylus sp* was recorded. *Moniezia* and algal cysts were the least prevalent. The eggs/cysts/oocysts per gram of feces (EPG/CPG/OPG) were also determined. The range of EPG/CPG/OPG varied among the parasites (0 to 600). Mean EPG count was highest in case of *Blantidium coli* (180±33.7) followed by that of *Buxtonella* (72±14.2), *Trichostrongylus sp.* (55.0±17.7), *Toxocora* (32.5±11.6), *Moniezia* (2.5±2.5). A low parasitic burden was found in case of algal cysts (0±00).

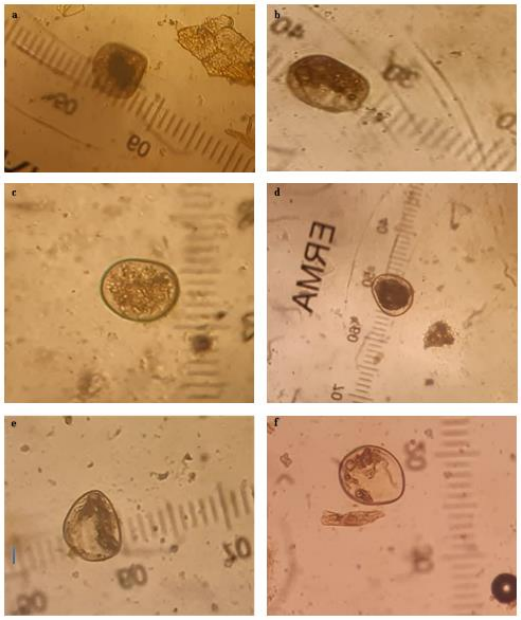

Figure 2: a) *Blantidium coli* 70x70 µm b) *Trichostrongylus sp*.70x60 µm c) *Toxocora sp*.70x60 µm d) *Buxtonela* 70x60 µm e) *Moniezia sp*.70x60 µm f) Algal cyst 70x60 µm

Table 1: Overall EPG of gastrointestinal parasites in hog deer

Parasites	EPG	Range	Mean±S.E	S.D
Blantidium coli	3600	0-600	180.0±33.7	150.78
Buxtonella	1400	0-150	72.5±14.2	63.81
Trichostrongylus sp.	1050	0-300	55.0±17.7	79.30
Toxocora sp.	650	0-150	32.5±11.6	51.99
Moniezia	50	0-50	2.5±2.5	11.18
Algal Cysts	0	0	0	0
Subtotal	6750	0-600		

3.1.2. Spotted deer

The EPG of parasites observed in spotted deer is provided in Table 2 *Blantidium coli*, *Buxtonella*, *Trichostrongylus sp*, *Toxocora sp*., *Moniezia* and algal cysts (4250, 18590, 650, 100, 300, 0 respectively). *Blantidiumc coli* was more persistent in spotted deer than *Buxtonella*. *Trichostrongylus sp* was moderately present whereas, *Monieziais* was even less common. *Toxocora sp* and algal cysts were present the least. The range of EPG/CPG/OPG varied among the parasites (0 to 650). Mean EPG count was highest in case of *Blantidium coli* (212±43.8) followed by that of *Buxtonella* (92.5±38.9), *Trichostrongylus sp*. (32.5±10.434), *Toxocora* (5.00±3.44) and *Moniezia* (12.50±6.15). A low parasitic burden was found in case of algal cysts (0±00).

Table 2: Overall EPG of gastrointestinal parasites in spotted deer

Names of parasites	EPG	Range	Mean±S.E	S.D
Blantidium coli	4250	0-650	212.5±43.8	195.92
Buxtonella	1850	0-600	92.5±38.9	174.17
Trichostrongylus.sp.	650	0-150	32.5±10.434	46.66
Toxocora sp.	100	0-50	5.00±3.44	15.38
Moniezia	300	0-100	12.50±6.15	27.50
Algal Cysts	0	0	0	0
Subtotal	7150	0-650		

3.1.3. Fallow deer

The EPG of *Blantidium Coli*, *Buxtonella*, *Trichostrongylus. sp*, *Toxocora sp*., *Moniezia* and algal cysts is given in Table 3 (2300, 500, 1300, 600, 800, 200 respectively). *Blantidium coli* was the most common (2300) followed by *Trichostrongylus sp* (1300) whereas the algal cyst were the least prevalent (200). The range of EPG/CPG/OPG varied among the parasites and ranged from 0 to 300. Mean EPG count was highest in case of *Blantidium coli* (115±20.5) followed by that of *Buxtonella* (25±6.7), *Trichostrongylus* sp. (65±12.61), *Toxocora* (30±9.17), *Moniezia* (40±16.05). A low parasitic burden was found in case of algal cysts (10±5.84).

Table 3: Overall EPG of gastrointestinal parasites in fallow deer

Names of parasites	EPG	Range	Mean ± SE	S.D
Blantidium coli	2300	0-300	115±20.5	91.9
Buxtonella	500	0-100	25±6.7	30.34
Trichostrongylus. sp	1300	0-150	65±12.61	56.42
Toxocora sp.	600	0-100	30±9.17	41.03
Moniezia	800	0-300	40±16.05	71.81
Algal Cysts	200	0-100	10±5.84	26.15
Subtotal	5700	0-300		

3.1.4. Red deer

Table 4 depicts the EPG of the *Blantidium coli*, *Buxtonella*, *Trichostrongylus. sp*, *Toxocora sp*., *Moniezia* and alga cysts (450, 350, 400, 0, 250, 400 respectively). *Blantidium coli* was the most common

(450) followed by *Trichostrongylus sp* (400) and algal cysts (400). Moderately prevailing parasite was *Buxtonella* (350) whereas *Moniezia* was less prevalent (250). *Toxocoro* was not observed in any of the Red deer samples. The range of EPG/CPG/OPG varied among the parasites from 0 to 100. Mean EPG count was highest in case of *Blantidium coli* (22.5±5.7) followed by that of *Buxtonella* (17.5±5.4), *Trichostrongylus sp.* (22.0±6.8), algal cysts (20.0±8.4), *Moniezia* (12.5±4.96) and *Toxocora sp.* (0.0±00).

Table 4: Overall EPG of gastrointestinal parasites in red deer

Names of parasites	EPG	Range	Mean ± S.E	S.D
Blantidium coli	450	0-50	22.5±5.7	25.52
Buxtonella	350	0-50	17.5±5.4	24.46
Trichostrongylus. sp	400	0-100	22.0±6.8	29.91
Toxocora sp.	0	0	0	0
Moniezia	250	0-50	12.5±4.96	22.21
Algal Cysts	400	0-100	20.0±8.4	37.69
Subtotal	1850	0-100		

3.1.5. Sambar deer

The EPG *Blantidium coli, Buxtonella, Trichostrongylus. sp, Toxocora sp., Moniezia* and alga cysts is provided in Table 5 (2050, 550, 650, 100, 200, 600 respectively).

Table 5: Overall EPG of gastrointestinal parasites in sambar deer

Names of parasites	EPG	Range	Mean ± SE	S.D
Blantidium coli	2050	0-600	102.5±35.07	156.83
Buxtonella	550	0-100	27.5±7.67	34.31
Trichostrongylus.sp	650	0-150	32.5±9.7	43.74
Toxocora sp.	100	0-50	5.0±3.44	15.39
Moniezia	200	0-50	10.0±4.5	20.50
Algal Cysts	600	0-100	30.0±7.6	34.02
Subtotal	4150	0-600		

Blantidium Coli was the most common parasite (2050) in Sambar deer. *Trichostrongylus sp*, algal cysts and *Buxtonella* were also widespread (550, 650, 600). Moderately prevailing parasite was *Moniezia* (200) however *Toxocora* was the least prevailing (100). The range of EPG/CPG/OPG varied among the parasites and ranged from 0 to 600. Mean EPG count was highest in case of *Blantidium coli* (102.5±35) followed by that of *Buxtonella* (27.5±7.67), *Trichostrongylus sp.* (32.5±9.7), algal cysts (30±7.6) *Moniezia* (10.0± 4.5) and *Toxocora sp.* (5.0±3.4).

3.2. Seasonal fluctuations in EPG of gastrointestinal parasites

Table 6 shows that seasonal fluctuations had a significant ($p<0.05$) effect on the EPG of gastrointestinal parasites in all deer species. Relatively higher frequency of gastrointestinal parasites were observed in winter than in autumn but the rate of change was not same for all parasites as it was noted that the EPG frequency of *Blantidium coli* increased (2500) in winters as compare to autumn (1100) in Hog deer. Similarly, *Buxtonella* (350-1050) and *Trichostrongylus sp.* (350-700) exhibited such tendencies. However, *Toxocora sp.* (100-100) remained same and *Moneizia* was reduced (50-0), algal cysts were not at all observed in Hog deer in both the seasons. In Spotted deer, the frequency of *Blantidium coli* and *Buxtonella* were high in winters as compared to autumn (1150-3100 and 200-1650). *Toxocora sp.* also increased in winter season (0-100). Presence of *Trichostrongylus sp.* and *Moniezia* were reduced (250-100 and 200-50). Algal cysts were not observed in both seasons. In Fallow deer the frequency of *Blantidium coli* (1600-700), *Buxtonella* (450-50), *Trichostrongylus sp.* (800-500), *Toxocora sp.* (300-300), *Moniezia* (250-550) and algal cysts (0.00 to 200) increased in winters. In Red deer frequency of *Blantidium coli, Trichostrongylus sp. Toxocora sp. Moniezia* and algal cyst decreased (300-150, 300-100, 0-0, 250-0, 400-0) in winters. Frequency of *Buxtonella* was increased (100-250). In Sambar deer prevalence rate of *Blantidium coli* and *Buxtenolla* was increased (400-1650 and 100-450) in winters as compared to autumn. Frequency of *Trichostrongylus sp,*

Toxocora, *Moniezia* and algal cysts decreased (650-0, 100-0, 200-0, 400-200).

Table 6: Seasonal fluctuations in EPG of gastrointestinal parasites

Name of parasite	EPG of gastrointestinal parasites									
	Autumn					Winter				
	*A	B	C	D	E	A	B	C	D	E
Blantidium coli	1100	1150	1600	300	400	2500	3100	700	150	1650
Buxtenolla	350	200	450	100	100	1050	1650	50	250	450
Trichostrongylus sp.	350	250	800	300	650	700	100	500	100	0
Toxocora sp.	100	0	300	0	100	550	100	300	0	0
Moniezia	50	200	250	250	200	0	50	550	0	0
Algal cyst	0	0	0	400	400	0	0	200	0	200

*A: Hog deer; B: Spotted deer; C: Fallow deer; D: Red deer; E: Sambar deer

3.2. Mean difference in EPG of gastrointestinal parasites

Statistical interpretation of collected data was used to determine that there were significant differences in EPG prevalence rate of gastrointestinal parasites *Blantidium coli*, *Buxtenolla*, *Trichostrongyluss sp*, *Toxocora sp*, *Moniezia* and algal cysts. Table 7 shows that the EPG of *Blantidium coli* was not significantly different (p=0.093/d.f=5/C.I=-271.5-2521.4) in Hog deer, Spotted deer, Fallow deer, Red deer and Sambar deer. There were no significant differences (p=0.139/d.f=6/C.I= -546.06-2912.7) in EPG of *Buxtenolla* parasites as well. The EPG of *Trichostrongylus sp*. was different in different species (p=0.051/d.f=5/C.I= -7.669-1745.66). EPG rate of *Toxocora sp*. was significantly different (p=0.005/d.f=5/C.I= 134.56-482.09) in Hog deer, Spotted deer, Fallow deer, Red deer and Sambar deer. Prevalence of *Moniezia* was significantly different (p=0.061/d.f=5/C.I= -45.46-1428.83) in Hog deer, Spotted deer, Fallow deer, Red deer and Sambar deer.

Table 7: Mean difference in EPG of gastrointestinal parasites

Name of parasite	Mean Difference in Gastrointestinal Parasites					
	T value	Degree of Freedom	Significance level p-Value	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Blantidium coli	2.071	5	.093	1125.000	-271.4791	2521.4791
Buxtenolla	1.759	5	.139	1183.333	-546.0610	2912.7277
Trichostrongylus sp.	2.548	5	.051	869.0000	-7.6689	1745.6689
Toxocora sp.	4.561	5	.006	308.3333	134.56	482.0987
Moniezia	2.412	5	.061	691.6667	-45.46	
Algal cyst	-	-	-	-	-	1428.8383

4. Discussion

Present study identified six gastrointestinal parasites *Blantidium coli*, *Buxtonella*, *Trichostrongylus. sp*, *Toxocora sp*., *Moniezia* and alga cysts in the feces collected from the captive Hog deer, Spotted deer, Fallow deer, Red deer and Sambar deer in Lahore zoo. Chambers *et al*. (2022) indicated that more parasite diversity results in more infections and higher mortality, and that the quantity of eggs/larva shed was likewise fairly constant throughout seasons. Betts *et al*. (2018) showed that a higher parasite variety resulted in far more infection and more death. Spotted deer of any sex, in any season, were discovered to be infected with several gastric and intestinal parasites without exhibiting any physiological symptoms of infection (Nayak *et al*., 2018). Several studies have previously noted that the spread of parasites may be impacted by season (Viljoen *et al*., 2011), temperature (Tinsley *et al*., 2011) and humidity (Altizer *et al*., 2006; Setchell *et al*., 2007). The source, exposure route, and host vulnerability make up the entire infection route. Certain parasites must become infectious larvae or need to be consumed by an intermediary host in order to infect the host. Maximum numbers of parasites infect mammals during the summer, indicating that the summer season offers the best circumstances for parasites to continue their life cycle (Dubey and Jenkins, 2018). Temperature greatly affects deer health as it allows more egg in faecal matter to hatch and increase growth rates, affecting their contagiousness (Goedknegt *et al*., 2015). Temperature and humidity are the two environmental parameters that have the greatest impact on the survival of free-living larvae on grasslands (Besier *et al*., 2016).

In current study, significant seasonal fluctuations (p<0.05) on the EPG of gastrointestinal parasitic were observed over a period of one year. Relatively higher frequencies of gastrointestinal

parasites are observed in winter than in autumn. These results were in accordance with findings by Shibitov and Abdelhamid (2022). The maximum parasite burden was likewise observed during the summer, indicating that the summertime infection load was extensive (Johnson and Hoverman, 2012). The development, transmission, and rates of infection of parasites are greatly influenced by rainfall and temperature (Altizer *et al*., 2006; Nieslen *et al*., 2007). Although summer is the plant kingdom's most productive season and a suitable time for deer to boost their diets, infectious spores remain on the grass, ready for the host to ingest it (Gruner and Cabaret, 1985). In other study (Tanjung, M. and Sibarani, 2018) the temperature compatibility, humidity, and oxygen availability were identified as only a few of the environmental factors outside the host body that have a significant impact on the emergence of worm infection.

The parasite eggs released with the excrement can hatch in favorable conditions and grow into infectious larvae that will infect the next host (Khatun *et al*., 2021). This implies that the likelihood of emerging worm infections will be higher in more favorable environmental conditions. External variables that support the parasite life cycle either directly or indirectly have a significant impact on the parasite's quality of life. The process of parasitism is directly related to a suitable environment for parasite development and reproduction along with susceptible hosts.

Present study revealed that the rate of *Trichostrongylus sp*. exhibited a unique trend as its rate significantly decreased in Spotted deer, Fallow deer, Red deer and Sambar deer. These results are consistent with the previous study (Woodhans *et al*, 2008) that suggested that summers are prolific season for *Trichostrongylus* to develop and it will decrease with decrease in temperature. The growing span of parasite populations can be expanded by an increase in the ambient temperature when it falls within the proper range.

In current work, *Toxocora sp* prevalence was found to be quite constant in Fallow deer however it increased in Hog and Spotted deer but decreased in Sambar deer. The rate or increase in *Toxocora sp* in winters is relevant with the previous study by Khatun *et al*. (2021).

Moniezia showed an equally unique trend as it decreased in winter, *Moniezia* EPG increased with increase in precipitation as the winters arrives the humidity decrease and cause decrease in its rate. These findings were in accordance with Gunn and Pitt (2022) that *Moniezia sp*. needs a warm environment to transform into cysticercoids inside of their intermediate host. Temperature affects how contagious parasite larvae are, and greater temperatures result in more cercarial formation (Goossens *et al*., 2015). *Moniezia sp* life's cycle requires a mite as an intermediate host therefore precipitation increases the rate of *Moniezia* prevalence substantially (Xu *et al*., 2021).

Algal cysts were constant in Hog and Spotted deer but increased in Fallow deer and decreased in Red and Sambar deers.

5. Conclusion

Findings from present study suggest that a further long term epidemiological study of gastrointestinal parasites is necessary for better understanding of parasitism in captive deers. It will surely ensure better management and adaptation of best possible ways of control, measure, and prevent the reoccurrence of existing infection in Hog deer, Spotted deer, Fallow deer, Red deer and Sambar deer. Results of current study can also be applied to other wildlife and zoonotic disease system of conservation and the public health concern and can assist the

clinicians for the prevention, diagnosis and control of such parasitic infections.

6. Acknowledgments

Authors acknowledge the administration of Lahore Zoo for their cooperation.

7. Author's Contribution

All authors contributed equally towards the conception, execution and write-up of current work.

8. Conflict of Interest

There are no conflicts.

9. Novelty Statement

The aim of the study was to determine the frequency and seasonal variations of major parasites *Blantidium coli*, *Buxtonella*, *Trichostrongylus sp*., *Toxocora sp*. and algal cysts in Hog deer, Spotted deer, Fallow deer, Red deer and Sambar deer in Lahore zoo. The study was conducted to improve our understanding of potential sources of pasture contamination.

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