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Molecular Diagnosis of Theileriosis and Associated Risk Factors in Captive Cervid and Antelope of Punjab, Pakistan

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Key words
Cervid, Antelope, Theileriosis, Sero-prevalence, Risk factors

ABSTRACT

Pakistan is home of a wide variety of animal species due to its four unique seasons. Numerous antelope and cervid species have their natural habitats in Pakistan's deserts, mountain ranges, along the Ravi River and Indus eco-regions. These species can also be found both in the wild and in captivity. We have to face great loses for both known and unknown reasons while keeping these species at different public and private zoological gardens. Hemoparasitic infections particularly theileriosis, transmitted by ticks, are one of the numerous recognized causes of their mortalities. To the best of our knowledge, no research has been done so far on the sero-prevalence and identification of associated risk factors for theileriosis in captive cervid and antelope in our study area. In the current study, the captive endemic species like *Axis porcinus*, *Antelope cervicapra*, *Gazelle g. bennettii*, *Ovis vignei*, *Boselaphus tragocamelus*, *Ovis orientalis orientalis and Rusa unicolor* as well as exotic species including *Axis axis*, *Dama dama* and *Addax nasomaculatus* of cervid and antelope being reared at eleven public and private zoological gardens were included. Out of n=200 samples, n=39 were found positive for Theileria spp through PCR by using 650bp sized primer pair on hypervariable region of 16S rRNA gene. The study also revealed that sex, age, type of enclosure, weather, body condition, chances of ectoparasite infestation and skin coat type were significantly associating with the occurrence of theileriosis in study animals. The study signified the diagnosis and treatment of tick-borne hemoparasitic diseases and proposed the PCR, an authentic technique of diagnosing theileriosis along with simple microscopy.

INTRODUCTION

The nilgai (*Boselaphus tragocamelus*), black buck (*Antilope cervicapra*), chinkara (*Gazella bennetti*), hog deer (*Axis porcinus*), and spotted deer/chittal (*Axis axis*) are among the five species of antelope, gazelle, and deer that have perished due to unknown causes, according to the Punjab Wildlife and Parks Department in Pakistan. To the best of our knowledge, no information has been published about the reasons for these ungulates' deaths in Pakistan (Ali *et al*., 2014) since Pakistan is a subtropical country in South Asia, ungulate mortality is largely attributed to hemoparasitic infections spread by many ticks. Infections transmitted by ticks pose a number of major health hazards to wild species of ungulates in captivity (Shahzad *et al*., 2013). Many different types of external parasites, including tick species (*Ixodes* spp.) have long been understood that they are important as a disease-carrying agent and a vector for the transmission of piroplasmosis (Randolph, 2000; Randolph *et al*., 2002).

The tick borne hemoparasites (TBHs), a group of pathogens that can infect both wild and domestic animal species (Ghai *et al*., 2016). Numerous pathogens, including protozoans with epidemiological implications for domestic animals and livestock, have been linked to deer across the globe (Huaman *et al*., 2021). Intraerythrocytic protozoans, such as the *theileria* genus, which are spread by hard-ticks of the Ixodidae family (Silveira *et al*., 2011) are not only

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found in cattle but also obviously infect wild animals (Li *et al*., 2016; Silveira *et al*., 2013).

Chronic high fever, weight loss, anemia, jaundice, and the risk of sudden mortality in young animals are among symptoms of *theileria* infections (Shahzad *et al*., 2013). The enlarged lymph nodes can be the pathognomonic sign of theileriosis. In addition to a sudden onset of fever and lethargy in the affected hosts, the infection may result in acute organ failure that may lead to death. However, infected animals can still carry the infection, commonly without displaying any clinical symptoms (Mosqueda *et al*., 2012; Solano-Gligo and Bennett, 2011).

Hemoparasites can easily be diagnosed primarily on the basis of clinical signs and routine microscopy. The subclinical stage of parasitemia, such as the trophozoite phase in blood, and the carrier stage cannot be detected by these conventional diagnostic techniques (Shahzad *et al*., 2013). The accurate status of hemoprastic infections among carrier animals is also determined using modern molecular diagnostic techniques, which have the capacity to overcome the limitations of conventional techniques employed for diagnosis, and effective management strategies for these diseases are developed. One technique that is frequently used to determine the exact species and assess the prevalence of *theileria* is PCR (Maharana *et al*., 2016). An 18S rRNA gene is most frequently amplified for phylogenetic study of *theileria* (Kazimirova *et al*., 2018).

Theileriosis, a tick-borne hemoparasitic disease, affects wild animals, especially captive ungulates, causing major losses in terms of their high cost and disturbances to natural ecosystem resources. There is a grey area in diagnosis of theileriosis in captive ungulates in Pakistan. So, the current study is specifically designed for ungulates in captivity and supervised by the Punjab Wildlife and Parks department. The current baseline study is designed to know the epidemiology and optimization of molecular diagnosis of theileriosis in captive ungulates of Punjab, Pakistan.

MATERIALS AND METHODS

Sampling site and study area

A total of 200 blood samples from captive ungulates (cervid and antelope) showing one or more clinical signs of disease from different public and private zoos and wildlife parks in fourteen districts of Punjab (Fig. 1), were obtained employing common minimally invasive capture methods.

Animal population

According to stock positions as of March 2022, a total of 1362 cervid and antelope were present 1 at sampling sites. Out of these 200 animals were selected randomly for sampling to screen out tick-borne hemoparasite i.e. *Theileria*. The species wise antelope and cervid populations at the captive sites in Punjab are given in Table I.

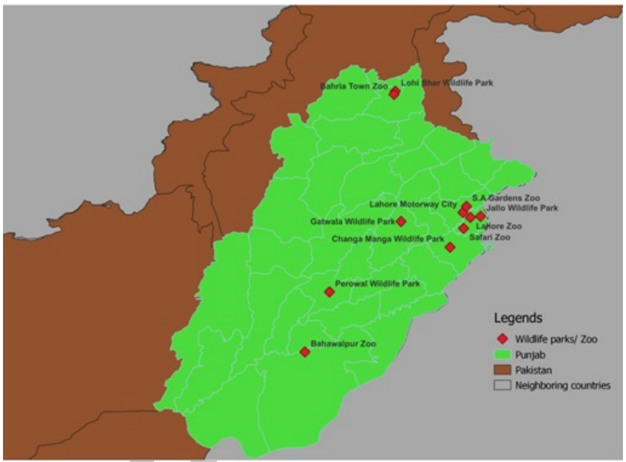

Fig. 1. Sampling districts of Punjab, Pakistan mentioned by red dots.

Inclusion criteria

The study included the exhibits that showing some or all of the clinical signs outlined here, including mild to high fever, anemia, anorexia, weakness, lethargy, swelling of the lymph nodes, rough hair coat, and jaundice, as well as a history of tick infestation. A data capture proforma was developed to record all of the details for each sample, including the sampling site, the animals, their management, and environmental conditions etc.

Sample collection and processing

Aseptic blood samples were obtained using 18-gauge disposable syringes from the left or right jugular veins of the animals with the help of stationed veterinarians and other related staff. According to Shrivastav (2011), the blood was then placed into a vacutainer coated with the anticoagulant ethylene diamine tetra acetic acid (EDTA) at a rate of 2 mg/ml. Moreover, thin blood smear slides were also made using aseptic ear tip puncture and slides were dried up on site to be used for microscopy.

Thin blood smears were made from all captive ungulates and stained with 10% Giemsa stain after fixation with methanol (Absolute) for 30 sec and were initially screened on the basis of microscopic examination under 100X oil immersion lens. The specimens were stated positive due to presence of inclusion bodies resembling hemoparasite i.e., *Theileria* (Ullah *et al*., 2022; Soulsby, 1982; Rehbein *et al*., 1998).

Table I. Species and sampling site wise positive cervid and antelope population.

Characteristics	Sampled animal n= 200	Microscopy positive n= 61	PCR positive n = 39	P value
Sampling location				0.6
Bahawalpur Zoo Bahawalpur	2	0 (0%)	0 (0%)	
Bahria Town Zoo Rawalpindi	7	2 (3.28%)	1 (2.6%)	
Lahore Motorway City Zoo	14	5 (8.20%)	4 (10%)	
Lahore Zoo Lahore	39	13 (21.3%)	8 (21%)	
S.A Garden Zoo Sheikhupura	13	3 (4.92%)	1 (2.6%)	
Safari Zoo Lahore	59	20 (32.79%)	14 (36%)	
Wildlife Park Changa Manga Kasur	18	9 (14.75%)	5 (13%)	
Wildlife Park Gatwala Faisalabad	13	0 (0%)	0 (0%)	
Wildlife Park Jallo Lahore	24	5 (8.20%)	4 (10%)	
Wildlife Park Lohi Bher Rawalpindi	3	0 (0%)	0 (0%)	
Wildlife Park Perowal Sahiwal	8	4 (6.56%)	2 (5.1%)	
Sampled animal species				0.8
Addax (*Addax nasomaculatus*)	3	2 (3.28%)	1 (2.6%)	
Black buck (*Antelope cervicapra*)	15	5 (8.20%)	3 (7.7%)	
Blue bull (*Boselaphus tragocamelus*)	3	1 (1.64%)	1 (2.6%)	
Chinkara (*Gazelle g. bennettii*)	10	4 (6.56%)	2 (5.1%)	
Fallow deer (*Dama dama*)	3	1 (1.64%)	1 (2.6%)	
Hog deer (*Axis porcinus*)	95	29 (47.54%)	18 (46%)	
Mouflon sheep (*Ovis orientalis orientalis*)	44	12 (19.67%)	6 (15%)	
Punjab urial (*Ovis vignei*)	10	3 (4.92%)	2 (5.1%)	
Samber deer (*Rusa unicolor*)	6	2 (3.28%)	2 (5.1%)	
Spotted deer (*Axis axis*)	11	3 (4.92%)	3 (7.7%)	

Molecular diagnosis

DNA was extracted using the "GeneAll ExpinTM Combo GP small, 200p" kit with Catalog No. 112-102. 40ng of DNA was used as the template for each amplification cycle in a reaction mixture of 20 µl containing 10 µl (2 times) PCR master buffers (Catalog No. W1401-2 wizbio solutions, Korea), 20 pmol of each primer (5'- AGTTTCTGACCTATCAG-3' as forward and 5'- TTGCCTTAAACTTCCTTG-3' as reverse primer for Theileria species self-designedwith 1098bp size on 18S hypervariable V4 region and 4 µl diethyl pyro-carbonate (DEPC) treated water (Catalog NO. 750023 Invitrogentm, USA). The reactions were carried out under the following conditions in a GS482 Thermocycler (G-STORM, UK).

For the *theileria* genus 95°C for five min, followed by 30 cycles of 95°C for one min and 56°C for 1 min and an extension step for 72°C for 10 min. The amplified products were analyzed using electrophoresis on 1.5% agarose gel. Gel electrophoresis was performed on 10 µl of each PCR product at 120 volts for 40 min. Westburg 1kb DNA ladder cat # BR BR0800101 was used to compare the PCR product's size. Primer product with a size of 1098 bp was regarded as positive for the genus *theileria*.

Associated risk factors

The suspected risk factors associated with hemoparasitic infestation were also studied along with prevalence of disease which were, species, sex, age, site of sampling, type and environment of enclosure, weather, other disease potential species in surrounding, in and out animals' movements, body condition of animal, chances of ectoparasite infestation and skin coat type etc.

Age of animals was assessed with the help of record available at the site along with physical characters, inspection of tooth replacement and wear of mandible teeth etc. Depending on the age, the animals were assigned their group as young (≤ 1 year) or adult (> 1 years). Keeping in view the physical appearance and tentative body weights, the physical condition of animal was also determined and scored as good, fair, emaciated or wounded etc. (Ali *et al*., 2014).

Statistic

al analysis

The prevalence was found simply by applying descriptive statistics and percentage formula while significance of associated risk factors was determined by using univariate analysis i.e. Chi-square or fisher exact test as per requirement. The association of risk factors with the occurrence of disease was developed through multivariate analysis i.e. Logistic regression. The whole data was analyzed by using R software. P<0.05 at 95% CI was considered significant and Odds ratio more than one was showing positive association while less than one as negative association with the occurrence of disease.

RESULTS

Prevalence of theileriosis

The sting-ring, oval, rod, or comma-shaped inclusion bodies in RBCs were considered as positive for theileria (Fig. 2). The 30.5% of the animals examined were found positive for *theileria*. Of the positive animals 47.54% were hog deer, 19.67% were mouflon sheep, 8.20% were black buck, 6.56% were chinkara, 4.92% were Punjab urial and spotted deer each, 3.28% were samber deer and 1.64% were blue bull, fallow deer and addax each (Table I).

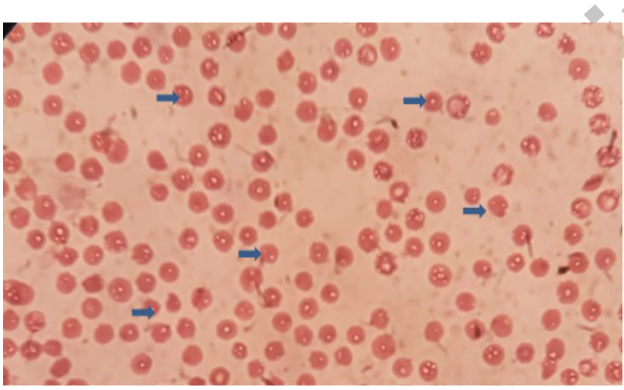

Fig. 2. Comma or signet ring shaped *Theileria* (Arrows) were seen in RBCs through thin blood slide at 100X (Giemsa staining).

From among the microscopically identified positive samples 63.93% were found PCR positive. Among the total PCR positive animals individually 46% were hog deer, 7.7% were black buck, 5.1% were chinkara, 15% were mouflon sheep, 5.1% were Punjab urial, 2.6% were blue bull, 7.7% were spotted deer, 2.6% were fallow deer, 5.1% were samber deer and 2.6% were addax (Table I).

The positive percentage of disease at various locations was found from highest to lowest as 36% at Safari Zoo Lahore followed by 21% at Lahore Zoo Lahore, 13% at Wildlife Park Changa Manga, Kasur, 10% each at Wildlife breeding Center Jallo, Lahore and Lahore Motorway City Zoo Lahore, 5.1% at Wildlife Park Perowal, Sahiwal, 2.6% each at S.A Gardens Zoo, Sheikhupura and Bahria Town Zoo, Rawalpindi and no positive case was recorded at Bahawalpur Zoo, Bahawalpur, Wildlife Park Lohi Bher, Rawalpindi and Wildlife Park Gatwala, Faisalabad (Table I). Whereas sampling site was not found significant factor (p=0.6) in univariate and multivariate analysis towards disease occurrence.

Associated risk factors

Table II shows the various risk factors associated with occurrence of *theileria* infection in captive cervid and antelope of Punjab, Pakistan. As far as the age of sampling animals was concerned, among the total positive animals, 87% including all species except chinkara were adult animals having age more than one year and 12.82% including 5.13% chinkara and 7.69% hog deer were young animals with age less than or equal to one year. Statistically, it was also found that age was not having significant role (p=0.6) in occurrence of disease. Similarly, the gender was also found a serious risk for *theileria* infection i.e. males were at higher risk than females, with overall rate 61.54% and 38.46%, respectively. Although gender was not found significantly (p=0.05) influencing factor in disease occurrence in univariate analysis.

The data regarding the type of diet offered to the animals at captive sites was also analyzed to find any significance in disease occurrence which was p=0.60 i.e. There was no role of type of diet in disease occurrence but the captive sites where green fodder plus concentrate was being offered as diet were found more positive 79% in *theileria* infection as compared to the green fodder diet 13% and pelleted diet 7.7%. The presence of ticks was found a significant factor (p=0.008) in causation of *theileria* infection in captive cervids and antelopes with odds ratio 10.55 (2.09-53.27) 0.004, which means presence of ticks has a strong relation with the occurrence of disease. The positive percentage 87% was found in presence of tick while 13% was found in case of absence of ticks.

The captive sites where acaricides were being used inside enclosure to stop the ectoparasite growth, those were having very low theileria infections 2.6% as compared to those where acaricides were not being used 97%. The p-value 0.03 1 was showing significance of the factor in disease occurrence. Similarly, the history of tick borne disease (TBD) was not found a significant factor in disease causation with p=0.8 while the treatment of tick borne disease was found highly significant (p<0.001) factor in occurrence of disease with less positive percentages 31% at TBD treatment history sites as compared to no TBD treatment history sites 69% (Table II).

Table II. Risk factors associated with occurrence of theileriosis in cervid and antelope.

Levels	Positive	Negative	p-Value	O.R	C.I	p-Value
Age of animal						
Young (≤ 1 Year)	5 (13%)	15 (9.3%)	0.6			
Adult (>1years)	34 (87%)	146 (91%)				
Sex of animal						
Female	15 (38%)	90 (56%)	0.050			
Male	24 (62%)	71 (44%)				
Diet of animal						
Green fodder	5 (13%)	24 (15%)	0.6			
Green fodder plus concentrate	31 (79%)	116 (72%)				
Pelleted feed	3 (7.7%)	21 (13%)				
Presence of ticks						
Present	34 (87%)	158 (98%)	0.008	10.54939	2.218171-62.95166	0.004348
Not present	5 (13%)	3 (1.9%)				
Use of acaricides						
No	38 (97%)	136 (84%)	0.031			
Yes	1 (2.6%)	25 (16%)				
History of TBD						
No	4 (10%)	14 (8.7%)	0.8			
Yes	35 (90%)	147 (91%)				
Treatment for TBD						
No	27 (69%)	38 (24%)	<0.001			
Yes	12 (31%)	123 (76%)				
Weather/Season						
Summer	25 (64%)	68 (42%)	0.068			
Fall	4 (10%)	26 (16%)				
Spring	8 (21%)	40 (25%)				
Winter	2 (5.1%)	27 (17%)				
Water for drinking						
Stagnant	4 (10%)	8 (5.0%)	0.3			
Tab	35 (90%)	153 (95%)				
Other species around						
Bovines	23 (59%)	61 (38%)	0.068			
Caprines	3 (7.7%)	25 (16%)				
Equines	0 (0%)	10 (6.2%)				
None	13 (33%)	65 (40%)				
Animal housing area						
Large cage	23 (59%)	67 (42%)	0.033			
Medium cage	3 (7.7%)	42 (26%)		5.320186	1.557079-25.5715	0.016176
Small cage	13 (33%)	52 (32%)		0.86408	0.36919-2.062054	0.737409

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Levels	Positive	Negative	p-Value	O.R	C.I	p-Value
Cracks/Tick hidings						
Not present	0 (0%)	31 (19%)	0.003			
Present	39 (100%)	130 (81%)				
Vector control program						
Not practiced	1 (2.6%)	25 (16%)	0.031			
Practiced	38 (97%)	136 (84%)		0.105208	0.005216-0.652494	0.046368
Quarantine for animals						
No	27 (69%)	125 (78%)	0.3			
Yes	12 (31%)	36 (22%)				
Body condition						
Normal	17 (44%)	95 (59%)	0.020			
Obese	0 (0%)	11 (6.8%)				
Weak	22 (56%)	55 (34%)				
Skin coat type						
Thick and hairy	31 (79%)	149 (93%)	0.032			
Thin and less hairy	8 (21%)	12 (7.5%)		0.174353	0.054317-0.539633	0.00245
In and out animal movement						
Frequent	29 (74%)	80 (50%)	0.021			
Less frequent	5 (13%)	45 (28%)				
No movements	5 (13%)	36 (22%)				

Fisher's exact test; Pearson's Chi-squared test.

The season was also found a substantial risk factor in occurrence of theileriosis. In summer season highest 64% positivity was recorded, followed by in spring 21%, in fall 10% and in winter 5.1%, whereas no role of weather/season in disease occurrence was determined in univariate analysis with p=0.068. The same was the case with drinking water for animals. Tab water was used by 90% positive animals whereas stagnant water was used by 10% positive animals with p=0.3 which was showing no relation with disease causation.

Similarly, the other tick host species around the captive sites as grazers was found to be a non-significant factor (p=0.068) in causation of disease while the captive sites where bovines were present as other tick host species as grazers were having more theileria infection positive cases 59% as compared to the sites where no grazers found around 33%, caprines as grazers 7.7% and equines as grazers 0%. The type and size of housing area was also found a significant factor for occurrence of theileria infection with p=0.033. But the odds ratio for medium sized housing area was determined as 5.32 (1.36-20.78) 0.016 and for small sized housing area as 0.86 (0.37-2.03) 0.737. Whereas the animals, which were being kept in large sized or safari type housing area were found at highest positivity of disease (59%) as compared to 33% in small and 7.7% in medium sized housing areas (Table II).

All the positive animals for theileriosis were from those enclosures where hiding sites like cracks were present. Statistically the role of cracks or tick hiding places in the enclosure was also found significant (p=0.003) in univariate analysis. While the vector control program was also found a significant risk factor towards disease occurrence with p=0.031 and odds ratio 0.11 (0.01-0.96) 0.046 which means vector control program has negative role in disease occurrence if practiced. The positive percentage at vector control program practicing sites was 2.6% as compared to non-practicing sites 97%.

The captive sites where quarantine protocol was practiced, 31% were positive which was less than the sites where quarantine protocol was not practiced (69%), while the p-value (p=0.30) which was showing no significant role of this factor in disease occurrence. Similarly, the body condition of animals was also found a significant (p=0.02) risk factor for occurrence of *theileria* infection. The animals with weak body condition were found highly affected (56%) followed by animals with normal body condition (44%) and no infection was found in obese animals (Table II).

The disease occurrence in species with thin less hairy skin coat was low (21%) compared to the species with thick hairy skin coat (79%). This character was found significantly influencing in disease occurrence with p-value 0.032 by univariate analysis and odds ratio 0.17 (0.06-0.54) 0.002 for thin less hairy coat. The in and out movements of animals at captive sites were also found significant (p=0.021) risk factors for occurrence of theileria infection with high positive percentage (74%) in frequent and 13% in each, less frequent and no movements sites as shown in Table II.

DISCUSSION

In various locations of Pakistan, studies have demonstrated the detrimental effects of *theileria* infection on livestock (Jabbar *et al*., 2015). Most of these investigations relied on microscopic diagnosis of the infection and lacked enough molecular data on the genetic diversity of prevalent *theileria* spp. (Mans *et al*., 2015). We believe that our study is the first of its type to provide a thorough assessment of the epidemiology, genetic basis for diagnosis, and factors associated for theileriosis in captive cervid and antelope in Punjab, Pakistan. *Theileria* species found to be prevalent in symptomatic captive cervid and antelope's blood samples through microscopic examination and PCR amplification of the 18S rRNA genus-based sequence. Significant correlations were found between the prevalence of theileriosis and a number of variables, like species, age, gender, location, enclosure type, ectoparasite risk, and different seasons of the year. It was found that molecular identification of *theileria* spp. in captive cervid and antelope was accurate and less ambiguous with high positive percentage, (63.93%) as compared to the microscopic diagnosis, (30.5%) as indicated in Table I.

A quick and inexpensive tool for identification and diagnosis of different infectious agents such as *theileria* spp., is microscopy (Mosqueda *et al*., 2012). Although, *theileria* species have been difficult to diagnose and precisely identify with this method. *Theileria* species that may exist singly or in combination with other species together within the same host may typically not be distinguished through microscopic analysis (Parthiban *et al*., 2010). Owing to cross reactive with other *theileria* species and the inability to distinguish between active carriers and animals with antibodies from earlier infections, serological assays like IFA and ELISA are therefore not effective (Dolan, 1986). But despite of all these limitations the traditional Giemsa stained thin blood smear examination proved to be the most acceptable method for identifying intra-erythrocytic piroplasm and the schizont stage of *theileria* spp., but it is rarely accurate when used with carriers (Durrani and Kamal, 2008).

Different species of ungulates and their enclosure type were found a substantial risk factor for theileriosis in captive cervids and antelope in Punjab, Pakistan. According to Kohli *et al*. (2014), the presence of carrier animals is cause for concern as it gets concentration towards infection to other predisposed healthy animals in the zoo, such as four horned antelopes, black buck, mouse deer, swamp deer, barking deer, hog deer, sambar deer, spotted deer, and brow-antlered deer. Additionally, he talked about how some animals that live in safari parks, such as barking deer, spotted deer, sambar deer, and four-horned antelopes, increase the chance of this parasite spreading to wild herbivores. The chronic type of piroplasm was usually belonged to the carrier animals. In addition, it was also evident that the male captive cervids and antelopes showed higher positive percentages than females. According to Naz *et al*. (2012), sheep males have a higher risk than females to contract theileriosis. During the mating season, male deer show higher stress levels than female deer, and these levels peak towards the end of the breeding season (USDA Report, 2002-2007). According to gender-specific data presented by Ali *et al*. (2014), males died most frequently (23.71%) from parasite infestation, followed by trauma (20.62%) while, among the parasites babesiosis and theileriosis were also identified (5.07%).

In the current investigation, adult animals of captive cervid and antelope (> 1 year) were more frequently observed to be infected with theileriosis than young ones (≤1 year). Swai *et al*. (2005) and Khan *et al*. (2022), also validated the findings of the study who found that adult animals (2 to 6 years old) had higher infection rates than young animals (≤2 years old). Adult animals may have a high infection rate because of their weakened immune systems and frequent exposure to foraging ticks. Early colostrum feeding in infants may improve immunity to a variety of infections, including *theileria* spp. (Rizk *et al*., 2017; Tabor *et al*., 2017). An inverse association between age and resistance to infection was identified by Garcia-Sanmartin *et al*. (2007). He found that fawns gradually develop immunity without displaying any clinical signs, and that immunity is retained by continuing to be exposed to the parasites. As a result, a long-lasting parasite reservoir develops in the wild ruminants. It is known that piroplasmosis, which is a severe illness and a leading cause of death in wild animals, can be brought on by various stressors such as high parasitemia, inadequate diet, high population density, harsh weather conditions, or mishandling during translocation (Hofle *et al*., 2004).

The captive sites included in the study where other tick host species were present for grazing, or captive species were having large sized enclosures were found highly infected with disease than those which were not exposed to other tick host species or having small sized enclosures. Swai *et al*. (2005, 2007), supported this point of this study by reveling that free-grazing animals had higher levels of *theileria* infection in open areas than in confined bounds. During free-grazing, it has been demonstrated that contact between healthy animals and infected or carrier host animals significantly increased the burden and transmission of *theileria* infection. If there is congestion or unsanitary environment, the small cages might also be a major risk factor for *theileria* infection. In a similar vein, livestock near the captive facilities may also be a potential source of this infection for the population of wildlife in the buffer zones (Daniels *et al*., 2007).

It was observed that in the current study season was also an important risk factor in the spread of *theileria* infection in captive cervids and antelope of Punjab, Pakistan. Highest positive percentages were observed in summer followed by spring, fall and winter. Kamran *et al*. (2021) and Ogden and Lindsay (2016) reported in their publication almost same findings that *theileria* infection rates were higher in the summer compared to the winter. Tick reproduction, growth, and distribution are favored by warm, humid weather; this may facilitate tick accessibility and search for hosts. Additionally noteworthy is the fact that the study area has had significant summer tick populations, particularly those that serve as vectors for *theileria* species.

CONCLUSIONS

The study concluded that hemoparasitic infections like theileriosis is prevalent in captive cervid and antelope of Punjab, Pakistan and there is dire need to device a comprehensive protocol to diagnose, treat and prevent this deadly disease so that heavy losses in the form of mortality may be avoided. The study also concluded that the associated risk factors like vector, its control and other related factors should also be addressed to coup up with the disease.

RECOMMENDATIONS

It is obvious that microscopy alone does not provide a confirmed and precise type of diagnosis, and that the etiological agent may not always be clearly identified. So, it is suggested that microscopic examination in combination with PCR should be adopted for the detection and accurate identification of *theileria* spp. The work could also be extended to all over Pakistan for the real sense domestic wildlife conservation.

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Ethical statement and IRB approval

The study was undertaken in compliance with the Institutional guidelines of Ethical Review Committee, University of Veterinary and Animal Sciences Lahore, Pakistan with No. DR/402 dated 30-9-2021 and no animal was harmed during study.

Statement of conflict of interest

The authors have declared no conflict of interest.

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