

Epidemiology of Tick Borne Haemoprotozoan Infection in Ruminants in District Peshawar, and Periphery, Khyber Pakhtunkhwa, (Pakistan)

Khurshaid Anwar^{a*}, Anwarud Din^b

^aSenior Research Officers, Veterinary Research & Disease Investigation Center Balogram, Swat, 19130, Pakistan

^bDepartment of Mathematics, Sun Yet-sen University, China

^aEmail: drkkhan06@yahoo.com

Abstract

Haemoprotozoans are responsible for considerable economic losses in livestock in Pakistan. In the present study 1101 blood samples (690 cattle (*Bos indicus*), 243 Buffaloes (*Bubalus bubalis*) 108 sheep, and 60 goats) were examined for blood parasites from June 2013 to June 2016. The overall prevalence of theileriosis in three years was found to be 67.39, 15.63, 15.74, and 0 % in cattle, buffaloes, sheep and goats, respectively. The prevalence of babesiosis in three years was found to be 15.79, 51.44, 14.81 and 41.66 % in cattle, buffalo, sheep and goats, respectively. The prevalence of anaplasmosis in three years was found to be 2.9, 13.99, 29.63, and 1.66 % in cattle, buffaloes, sheep and goats respectively. The highest prevalence of theileriosis, babesiosis and anaplasmosis was recorded in cattle, buffaloes and sheep respectively, while goats showed negligible small prevalence percentage of anaplasmosis and Zero % of theileriosis.

Key Words: Haemoprotozoans; livestock; buffalo; theileriosis; babesiosis; anaplasmosis; prevalence.

1. Introduction

The haemoparasitic diseases are caused by vector borne blood parasites (haemoprotozoans), constitutes a disease entity of considerable economic importance in tropic and subtropics [31].

* Corresponding author.

These diseases are major limiting factors in maintaining exotic, cross-bred cattle, local cattle breeds, buffaloes and small ruminants in these areas. Haemoparasites bring losses to animals in terms of morbidity and mortality directly and indirectly economic and production losses due to their heavy incidence [8]. These diseases are now-a-days very common in all the tropical and subtropical zones of the globe. The areas where people care less for management, ticks and flies control face many haemoparasitic disease problems in their livestock and bears heavy economical and productive losses. Haemoprotezoa like *Theileria*, *Babesia* and *Anaplasma* are considered to be the most important blood parasites of cattle and buffaloes in Pakistan. Sporadic cases of the diseases caused by these three protozoa are seen throughout the year [2; 22]. However, their Outbreak in exotic and crossbred cattle is mostly reported during the hot and humid months (July, September) of the year. Occurrences of these parasites have been reported by [7] and [6] in apparently healthy cattle and by [3] in both cattle and buffaloes. [22] recorded 112 cases of theileriosis between March, 1993 and September, 1998 at Faisalabad and recommended further work on its sub-clinical infections. *Babesia* is intraerythrocytic parasites of domestic animals and is the cause of anemia and haemoglobinuria. They are transmitted by ticks in which the protozoan passes trans-ovarially, via the egg from one ticks generation to the next. The disease babesiosis, is particularly severe in naive animals, introduced into endemic area, is a considerable constraint on livestock development in many parts of the world. Babesiosis is also called Tick Fever disease or piroplasmosis. Hard ticks of the family Ixodidae in which trans-ovarian infection ensures that *babesia* is transmitted by stages of the next generation of ticks [30]. A group of *Babesia* spp. i.e. *B.bovis*, *B.bijemina*, *B.ovis* responsible for tick-borne diseases also called piroplasmosis. It seems probable that these diseases and the ticks responsible transmit due to transcontinental shipments of cattle. Cross-strain immunity is quite strong in *B.bovis* and a single strain of *B. bovis* has been used as vaccine Cross immunity confers protection to all *babesia* infection. The pathology of this disease is due to the rapidly dividing parasites in RBC's produce rapid destruction of the erythrocytes resulting haemoglobinemia, haemoglobinuria and fever. The acute cases may cause death in 20% of the infected animals, parasitemia may involve between 0.2% and 45% of the RBC's [30]. Bovine anaplasmosis or gall sickness caused by the intraerythrocytic rickettsia, *Anaplasma marginale* (Rickettsiales: Anaplasmataceae) is transmitted biologically by infected ticks or mechanically by biting flies and contaminated fomites [26]. Acute anaplasmosis is characterized by fever, progressive haemolytic anemia, weight loss, abortion and even death resulting in significant loss to meat and milk production [1]. The economic loss due to infections caused by *Babesia* and *Anaplasma* infections in India was estimated to be \$57 million [29]. The genus *Anaplasma* also includes *Anaplasma bovis*, formerly referred as Ehrlichia bovis [5]. Theileriosis is also a tick-borne disease. *Boophilus microplus* a tick responsible as a vector for this disease. The disease shows no composite clinical regime. *T.parva* and *T.annulata* sometimes *T.mutan* are important spp. responsible for theileriosis in cattle and small calf. This parasite causes symptoms of depression, elevated temperature, swollen peripheral glandular structures, lacrimation and labored breathing at terminal stages of the disease. *Theileria* organism remains permanently in blood stream and ticks get infected during blood sucking from previously infected cattle which have been infested by these ticks. This disease also occurs in areas where ticks *B. microplus* is not present indicates that some other vectors are also responsible for dissemination of this disease. Anaplasmosis is an infectious disease of cattle, dogs and buffaloes, characterized by anemia and caused by the parasite of the RBC's *Anaplasma marginale* and *Anaplasma centrale*. This parasite is cosmopolitan in nature, the disease co-exists with babesiosis, but pure infection may also occur. This disease is characterized by acute anemia, fever,

jaundice and degeneration of the internal organs. No RBC's destruction at fast level occur, therefore hemoglobinuria is not seen clinically. Naturally buffaloes, bison, various antelopes, deers, elks, camels, goats and sheep are all susceptible to anaplasmosis. Prenatally *A. marginal* may transmit when the dam is undergoing her primary infection. *Anaplasma*, *babesia* and *theileria* are the most important and heavily prevalent blood protozoans of cattle, buffalo, sheep and dogs in Pakistan. The diseases caused by these blood protozoans are known as anaplasmosis, babesiosis, and theileriosis, respectively [2].

These diseases are tick-borne because most of the transmission and dissemination of these diseases occurs via numerous genera of tick vectors [21]. Occurrence of these three hemoparasites are noted sporadically and cause disease throughout the year [2; 22; 7; 6] reported these hemoparasitic diseases in apparently healthy cattle and both in cattle, buffaloes and small ruminants specially sheep and goats [3]. In recent study conducted by [16] stated that the prevalence of theileriosis in cattle in D.I. Khan, Tank and Bannu was 51.24 and 26% respectively. The present study, therefore, was designed to determine the prevalence of haemoprotozoans in cattle, buffalo and small ruminants in District Peshawar and its periphery in Khyber Pakhtunkhwa, Pakistan.

2. Materials and Methods

This study was conducted at the Parasitology Division, Directorate of Veterinary Research Institute, Peshawar NWFP (Khyber Pakhtunkhwa). The study was conducted on random blood smears being prepared on the spot by the technical staff of the concerned division or by the field veterinarians. The farmers visited this section; mostly belong to districts Peshawar, Nowshera, Charsadda, and Mardan. Samples of some Afghan nomads' flocks, Military, government dairy farms and some cases of Khyber Pakhtunkhwa Agricultural university Peshawar dairy farm were also incorporated in this study. A total of 1101 samples were examined for the purpose to determine the prevalence of hemoprotozoans in ruminants from June 2013 to June 2016. Out of these, 1101 samples examined for hemoparasitic disease diagnosis, in which 690 were from cattle, 243 buffaloes, 108 sheep, and 60 goats respectively. Most of the slides were referred by the field veterinarians on the basis of their field experience and clinical signs of the diseased animals. Blood smears were made for detection of blood protozoans under microscope.

The blood smears were prepared aseptically on clean and grease free sterile glass slides directly from the blood collected mostly from ear vein, jugular vein or mammary vein. Each smear was dried in air, fixed with methanol and then Geimsa stain was used to stain the blood smear. Stock solution of Geimsa stain was diluted with distilled water in 1:10 ratio and double filtered by using Watt man's filter paper. Blood slides were then flooded with stain solution for 25-30 minutes. The slides were washed with tap water to remove the extra stain and allowed to air dry and examined under oil immersion objectives, using the standard procedure by [18]. The animals included in this study were not vaccinated against any blood protozoans nor categorized according to age, sex and lactation period.

2.1 Statistical Analysis

The data was analyzed by simple percentage method.

3. Results and Discussion

A total of 1101 blood smears of cattle, buffalo, sheep and goats from Peshawar and surrounding areas were examined. Out of 1101 samples, 882 were found positive for three genera of the heamoprotozoans i.e. *Theileria*, *Babesia* and *Anaplasma*. The overall infection prevalence percentage of theileriosis in three years was found to be 67.39, 15.63, 15.74 and 0% in cattle, buffalo, sheep and goats respectively. (Table1-4)

Table 1: Prevalence of Different Heamoprotozoans in Cattle in District Peshawar, and Periphery, Khyber Pakhtunkhwa, (Pakistan).

Year	Total Cases	Cases Positive for Haemoprotozoans			Total Prevalence
		Theileriosis	Babesiosis	Anaplasmosis	
2013-14	195	115 (58.97%)	27 (13.85%)	8 (4.1%)	76.92%
2014-15	235	170 (72.34%)	30 (12.77%)	4 (1.7%)	86.81%
2015-16	260	180 (69.23%)	52 (20%)	8 (3.08%)	92.30%
Total	690	465 (67.39%)	109 (15.79%)	20 (2.9%)	86.08%

Table 2: Prevalence of Different Heamoprotozoans in Buffaloes in District Peshawar, and Periphery, Khyber Pakhtunkhwa, (Pakistan).

Year	Total Cases	Cases Positive for Haemoprotozoans			Total Prevalence
		Theileriosis	Babesiosis	Anaplasmosis	
2013-14	77	10 (12.99%)	39 (50.65%)	7 (9.09%)	72.73%
2014-15	63	7 (11.11%)	40 (63.49%)	10 (15.87%)	90.48%
2015-16	103	21 (20.39%)	46 (44.66%)	17 (16.50%)	81.55%
Total	243	38 (15.63%)	125 (51.44%)	34 (13.99%)	81.06%

Table 3: Prevalence of Different Heamoprotozoans in Sheep in District Peshawar, and Periphery, Khyber Pakhtunkhwa, (Pakistan).

Year	Total Cases	Cases Positive for Haemoprotozoans			Total Prevalence
		Theileriosis	Babesiosis	Anaplasmosis	
2013-14	37	4 (10.81%)	9 (24.32%)	12 (32.43%)	67.57%
2014-15	49	7 (14.29%)	5 (10.20%)	11 (22.45%)	46.94%
2015-16	22	6 (27.27%)	2 (9.09%)	9 (40.90%)	77.27%
Total	108	17 (15.74%)	16 (14.81%)	32 (29.63%)	60.18%

Table 4: Prevalence of Different Haemoprotozoans in Goat in District Peshawar, and Periphery, Khyber Pakhtunkhwa, (Pakistan).

Cases Positive for Haemoprotozoans					
Year	Total Cases	Theileriosis	Babesiosis	Anaplasmosis	Total Prevalence
2013-14	21	0 (0%)	8 (38.1%)	1 (4.76%)	42.86%
2014-15	23	0 (0%)	11 (47.83%)	0 (0%)	47.83%
2015-16	16	0 (0%)	6 (37.5%)	0 (0%)	37.5%
Total	60	0 (0%)	25 (41.66%)	1 (1.66%)	43.33%

Table 5: Overall Prevalence of Haemoprotozoans in Buffaloes, Cattle, Sheep and Goats in District Peshawar, and Periphery, Khyber Pakhtunkhwa, (Pakistan).

Haemoprotozoans (Theileria, Babesia and Anaplasma) in Buffaloes, Cattle, Sheep and Goats			
Year	Total Examined Cases	Total Positive Cases	Overall Prevalence
2013-2016	1101	882	80.01%

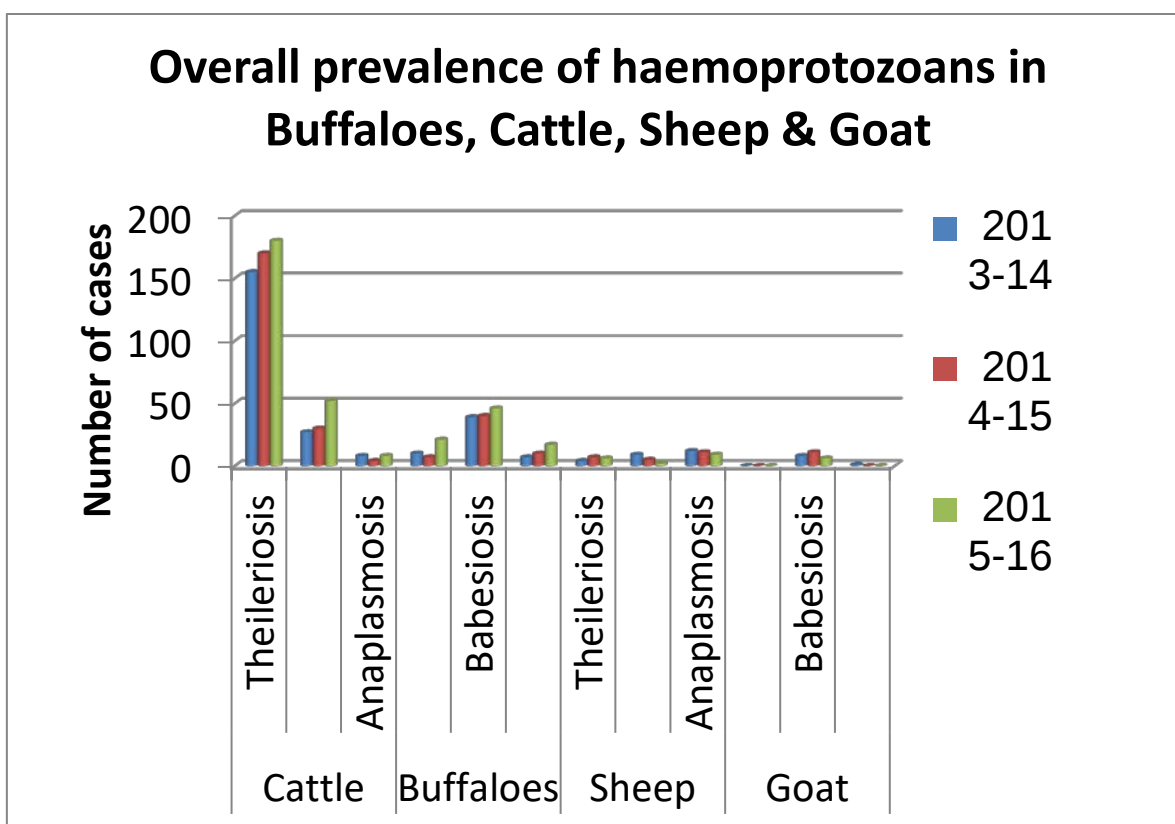


Figure 1

The aggregate prevalence percentage of babesiosis in three years was found to be 15.79, 51.44, 14.81 and 41.66 % in cattle, buffaloes, sheep and goats respectively (Table 1-4). The aggregate prevalence percentage of anaplasmosis in three years was found to be 2.9, 13.99, 29.63 and 1.66% in cattle, buffaloes, sheep and goats respectively (Table 1-4). The lowest prevalence in three years recorded for theileriosis in goats was zero percent (Table 4). This study does not agree with the findings recorded by the [20] Mayzyad and his colleagues (2002) they recorded 7.1 and 7.5% babesiosis and thelariosis in sheep and goats, respectively. In the same study they recorded 9.6 % and 8.1% babesiosis and thelariosis respectively in cattle. It might be concluded that the finding of our study disagree with the study of [20] may be due to their restricted area of consideration for the animals observed and some epidemiological factors important for haemoprotozoan diseases. In the rest of the species the values were recorded are of considerable importance and showed that highest prevalence of babesiosis occurred in Buffaloes 51.44% (Table 2) and the second highest values followed by theileriosis in cattle 67.39% (Table 1), respectively. The present study was mostly conducted during February, March and April, the favorable period for ticks' infestation. In Pakistan, the native breeds of cattle and buffaloes are resistant to ticks. So Pakistan villagers usually do not worry much about ticks' infestation. The present findings revealed that tick carrying sheep had higher percentage (29.63%) of prevalence of the parasite than buffaloes (13.99%) (Table 3 & 2). Because cattle are the main hosts of *Anaplasma* parasite [19] and although buffaloes are susceptible to most of the diseases and parasites that afflict cattle, the effect is often less serious than that on cattle in same ecosystems. Water buffaloes are generally considered as healthier animal in comparison to cattle [15]. The present finding on the occurrence frequency of ticks' infestation in cattle and buffaloes is 2.9% & 13.99% (Table 1 & 2) which is not in accordance with the findings of [14], as their result was 61% in Karachi and adjoining areas. This difference may be due to the moderate climate and high relative humidity in Karachi that favor ticks' infestation. In the present study, the prevailing percentage of parasite in buffaloes was 81.06% (Table-2) which is not in accordance with the study of [3] conducted in the same area showing that 30% buffaloes had infection. [24] found 11.8% prevalence of *A. marginale* in cattle of Sorti District, Uganda, while in the present study it was 2.9% (Table-1) this contrast in both the studies might be due to the poor managemental practices in Ugandas. This study is in partial agreement with the study conducted by [32], they reported 8.06% and 8.77% anaplasmosis and babesiosis only in cattle spp. respectively. Although microscopy is widely accepted and cost effective technique for the diagnosis of haemoprotozoans and haemoreticidal organisms, the technique lacks the higher sensitivity. The low prevalence observed in the study carried by Ristic and his team may be due to the fact that carrier cattle usually do not reveal inclusions in their blood films [26]. The findings in the study of Gale indicated the higher sensitivity and specificity of the molecular methods [9; 10] compared to the conventional methods especially in detecting low level rickettsaemia seen in carrier animals. Microscopy could not detect a single case of *A. bovis* in the study of [28; 27] could detect 0.53 per cent out of 150 blood smears collected from Chennai. [28] couldn't detect *A. bovis* in a blood smear of an infected buffalo and later identified the etiology by culturing the organism in blood mononuclear cells. All the above studies are in contradiction with our findings in which 0-34% *A. bovis* was detected in blood smears of infected animals. This might be due to high infestation and on time detection of the haemoprotozoans under the microscope and might be due to our year wise survey work on anaplasmosis in cattle as compared to their studies' findings. In the Kenya highlands [4; 11; 24; 25; 12; 13] have demonstrated that the prevalence of *Theileria parva* infections and the reported East Coast Fever morbidity, mortality and case-fatality can vary significantly by zones and grazing

system and that these differences have important implications for both the impact and control of East Coast Fever. The present study is in agreement with the epidemiological survey conducted by [17] in cloven footed animals in Khyber Pakhtunkhwa; who reported 44.27 and 27.11 % babesiosis and theileriosis respectively in cloven footed animals. The aggregate infection percentage of three years for babesiosis in cattle was 15.79 and anaplasmosis 2.9%, respectively. The aggregate infection percentage for babesiosis and anaplasmosis was 51.44 and 13.99 %, in buffaloes while it was 15.63% for theileriosis. In sheep the aggregate percentage of infection prevalence recorded for theileriosis was 15.74 %, babesiosis 14.81 % and anaplasmosis was 29.63 % respectively, which was the highest prevalence percentage of infection in all the spp. for anaplasmosis. The aggregate infection percentage of babesiosis and anaplasmosis in goats was 41.66 and 1.66 %, respectively (Table 4). The data in the study of [23] clearly suggested the higher sensitivity of molecular techniques for diagnosis of these diseases and in the present study available simple resources were used.

The incidence of babesiosis infection in goats was second to highest in buffalo (41.66 %) as shown in (Table 4). The findings of the study revealed that, the prevalence of different blood protozan infection was different in all the four animal species and there is no co-relation of infection prevalence among the different species. The lowest prevalence of theileriosis recorded in goats may be due to the facts that most of the flock owners do not care for their small ruminants and the animals expire without any laboratory diagnosis. Another reason might be that the low presence of vector ticks in goats may be responsible for low prevalence of theileriosis infection. However, this need more comprehensive study, specially focused on the caprine species. The pattern of the present study was different from all other studies conducted so far in this regard. This study encircles four different animal species and three blood parasites, so the incidence percentages for different hemoparasitic infection cannot be correlated with the studies being conducted on one animal *spp.* or one blood protozoan.

4. Conclusions

Parasitological survey is useful for a number of reasons: a/ to detect parasites; b/ to reveal the area of huge infestation caused by migrating larval forms and mature parasites; c/ to apply appropriate treatment; d/ to explain why certain treatments are not effective during a parasite invasion (parasites damage parenchymal organs causing permanent organ dysfunction and inducing formation of fibrotic scars). Cattle, buffaloes and small ruminants in Peshawar and suburbs were infected with helminths and other worms. The prevalence rates of GI parasites varied with month. The EPG of infection was moderate in most animals warranting treatment. Future studies are required to evaluate the economic impact of GI parasites in the study area.

5. Recommendations

This study recommends proper steps to be taken by the Livestock Department, including farmers' awareness through mass media campaign for tick control measures, acaricidal spray through arranging field days, timely laboratory diagnosis of protozoan diseases and close contact between farmers and Veterinary Research Institutes.

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Competing Interests

The authors declare that they have no competing interests.

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