

Epidemiology of Haemoprotozoal Infection of Small Ruminants in Surrounding Region of Arid Zone Research Institute of District Kohat, Pakistan

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Abstract | Haemoprotozoan cause considerable complications in domestic animals which consequence to huge economic losses. The present study was planned to determine the prevalence of haemoprotozoa of small ruminants in arid zone small ruminant research institute kohat from July 2017 to June 2021. The prevalence was studied in relation to species, seasons and different housing system or herds. Random blood samples were received and collected from the surrounding village of arid zone research institute kohat. A total of 726 blood sample were analyzed from both sheep and goat. Out of the total blood sample (62.39%) total prevalence were recorded for all type of hemoprotozoa. In which highest prevalence was recorded for theleriosis 46.13%, followed by anaplasmosis 29.13%, mixed infection 17.66% while the lowest prevalence was recorded for babesiosis which is 7.06%. Seasonal wise overall prevalence was recorded highest in monsoon 48.69%, 52.46% followed by summer 34.34%, 30.49% and winter 16.95%, 17.04% for sheep and goat respectively. High prevalence of hemoprotozoan was recorded in unorganized farm as compared to organized farm for both species' sheep and goats. The present study provides data of hemoprotozoa of small ruminant of arid zone research institute kohat and surrounding villages which will help in the eradication and other adoptive measure for prevention and control against blood protozoal parasite.

Keywords | Epidemiology, Haemoprotozoan, Small ruminants, Arid zone, Kohat.

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INTRODUCTION

Livestock plays a backbone and fundamental role in the basic domestic demand of meat, milk, mutton, hides and other different types of byproducts. More than 15 million farmers involve with this profession. Livestock contribute 61.89 % in agriculture and 14.04 % in GDP which recorded a growth of 3.26 %. (ESP 2021-22). Demand for Small ruminant especially due to their fast-growing capability, economic and less hectic farming increasing day by day. Preference for mutton and Chevon over beef meat is another major reason for increased demand and produc-

tion of small ruminants in Pakistan (Abubakar et al.,2015). But there are several elements and factors which badly affect the sustainable, productivity and profitability of these small ruminant in which parasite play the uppermost role (Ghosh et al., 2018). In successful farming all over the world parasitism is the single most big issue and hurdle (Saeed at al., 2010). Parasite specially hemoprotozoan encourages severe morbidity which eventually diminishing milk and meat production and lead to mortality which cause massive Economic losses and badly effect the farming profitability. (Ali et al., 2022; Ananda et al., 2009). Sheep and goats are extremely exposed to wide range of parasitic infections mostly haemoparasite which cause

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Theileriosis Babesiosis and Anaplasmosis which are listed in top 10 economical destructive diseases of livestock (Maske et al., 1990). Haemoprotozan is a big challenge to livestock farmer particularly in all hot and humid zones of the world (Ramadhas, 2005).

Ticks are the basic carrier of hemoprotozoan in livestock mainly in tropical and subtropical territories of the world (Soulsby, 1982). It is revealed from geographical distribution that Pakistan is a tropical country which provides optimal environment for production, reproduction, and multiplication of ticks. Ticks destroyed the animal health and production by sucking blood in huge amount and transmit several different viral, protozoal, and rickettsial diseases (Soulsby, 1982). An adult female tick may suck 0.5 to 2 ml of blood from animal (Pegram and Chizyuka, 1990). So, If an animal carries abundant or several ticks then it will lead to significant loss of blood which is the clear cause of decreased productivity, death and ultimately negative impact on livestock economic (Uilenberg, 1995).

In Pakistan especially arid zones haemoprotozoan infections, particularly Theileriosis, Babesiosis, and Anaplasmosis are considered severe hurdles to the physical condition and productivity of livestock (Rajput et al., 2005). As mentioned above that the hot and humid climate of Pakistan provided the ideal environment to tick devolvement, breeding and survival. (Kohli et al., 2014).

Theileriosis exhibit the Clinical signs which include anorexia, dull, depressed, lacrimation, and most common swelling of superficial prescapular lymphnodes, enlargement of liver, spleen, and icterus (Naz et al., 2012). *Theileria ovis* and *Theileria hirci* (*lestoquardi*) are the important species of theileria which cause infection in sheep and goats (Taylor et al., 2016). *Theileria lestoquardi* and *T. ovis* are rod like, spherical, ovoid are irregularly shaped organisms found mostly in lymphocytes, erythrocytes and histiocytes which lead to theileriosis (Khan et al., 2017; Nejash and Tilahun, 2016; Gul et al., 2015; El-Azazy et al., 2001).

Babesiosis show signs of hemoglobinuria, anemia, and high body temperature clinically *Babesia ovis* is common species which is transmitted from one animal to other animal by ticks specially Rhiphicephalus species in sheep and goats only (Shah et al., 2017). *Babesia ovis* and *B. motasi* is spherical to pyriform with an amoeboid form intra-erythrocytic protozoan parasite and cause economical losses in the small ruminants in the form of babesiosis (Taylor et al., 2016).

Anaplasmosis exhibit clinical signs of anorexia, jaundice, anemia, fever, abnormal signs related to respiratory system and decline in body weight and milk production. Sometime reproductive signs also exhibited with anaplasmosis. Anaplasma centrale, Anaplasma marginale and A. phagocytophilum are common in small ruminants (Razmi et al., 2006; Garcia-Perez et al., 2003). Similarly, to other blood parasite this is also causes huge economic losses due to productive and reproductive losses (Matsumoto et al., 2006).

Pathology of the above mentioned haemoprotozoal disease is that these blood parasites destruct the erythrocytes due to rapid division in red blood cell which results in fever and jaundice. Death up to 20% are also recorded in acute cases of animal suffering from anaplasmosis. (Said et al., 2015).

For the prevention and control of the above mentioned terrible hemoprotozoal diseases an appropriate knowledge of the epidemiology measures is an essential tool to design a control strategy. There is very less work done on the prevalence of this blood parasite on this specific area of kohat division. Hence, the present study was design to investigate the seasonal and species wise prevalence and risk factor of Theileriosis, Babesiosis, Anaplasmosis, and mixed infection of haemoprotozoan in small ruminants of this specific area of kohat division (Pakistan).

MATERIALS AND METHODS

STUDY AREA

This study was conducted at the laboratory section of Arid Zone Small Ruminant Research Institute Ghulam Banda Kohat (Khyber Pakhtunkhwa). Mostly sample collected from surrounding villages of azsrri i.e Ghulam banda, shen dhand, babri banda and ghumbat.

The basic tool and instruments used for blood sample screening and processing were syringe, needle, Icebox, Ethylenediamine tetra acetic acid (EDTA) tube, hand gloves, clean glass slide and cover slip, tissue paper, beaker, pipette, oil immersion, distilled water, Giemsa stain10%, absolute methanol, buffer solution and Microscope. Mostly blood collected from ear vein due to high chances of hemomprotozan which mostly trapped in vein and collected from jugular vein and thin smears prepared directly after collection of blood by the laboratory technical staff from the experimental station animals. Blood samples were also collected from sheep and goats from out station rural area farmers. Properly packed Ice box were used for transportation of outdoor blood sample to laboratory section of AZSRRI for further examination.

Livestock especially sheep and goat in the selected villages were mostly rising in intensive and semi-intensive system mean organized and less organized farming system.

SAMPLING NUMBER AND DURATION

The sample collected and analyzed in the period between

July 2017 to June 2021

A total of 726 sheep and goat were randomly investigated from the above-mentioned villages. Out of 726 small ruminants 388 goats and 338 sheep were examine and investigated for hemoprotozoan. The prevalence research was noted based on species, season and housing systems. Three most common seasons were selected that was Rainy Monsoon (July–October), summer (April–June), and winter (November–March).

PREPARATION OF THIN BLOOD SMEAR AND EXAMINATION

After arrival of the blood sample to the lab in ice box, EDTA tube having anticoagulated blood sample were opened gradually and thin blood smear were prepared and were stained with Giemsa 10% following standard protocol. With the help of micropipette one drop of blood was collected from EDTA tube and placed on the clean slides and thin smears were prepared with the help of another clean slide. The smears were fixed with the help of methanol for 5 minutes. The fixed slide was stained with Giemsa 10% for 20-25 min. after staining all the slide were washed under slow running tap water. Let them all slides air dry for few minutes and examined under microscope. Oil immersion lens having (×100 magnification) were used for the detection of haemoprotozoa (Soulsby, 1982). The parasites were recognized based on characteristics, shape and morphology (Taylor et al., 2016; Soulsby, 1982).

STATISTICAL ANALYSIS

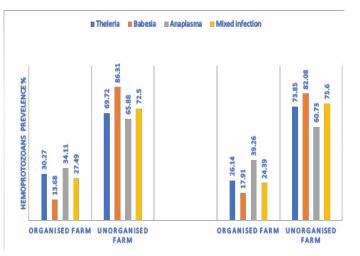
Data collected and was arranged in MS Office Excel and Statistical analysis was carried out by the simple percentage method.

RESULTS

In the present study the prevalence of haemoprotozoan parasites in small ruminants in kohat for the period from July 2017 to June 2021 were recorded. Prevalence was recorded on different aspects such as prevalence in sheep, prevalence in goats, overall prevalence, prevalence among different season/weather and prevalence of different small ruminants housing system.

PREVALENCE OF DIFFERENT HEAMOPROTOZOANS IN SHEEP

A total of 338 blood smear investigated for the examination of hemoprotozoan in which 230 were recorded positive for hemoprotozoans. It's showed that the total prevalence is (68.04%) for the period of 04 year in sheep. Among the hemoprotozoan highest prevalence was recorded for Theileriosis which is (47.82%) followed by Anaplasmosis (29.13%), Mixed infection (15.65%) while lowest preva-



lence was recorded for babesiosis that is (7.39%). Table 1

Figure 1: 1) Sheep housing types, 2) Goat housing types.

PREVALENCE OF DIFFERENT HEAMOPROTOZOANS IN GOATS

A total of 388 blood smear investigated for the examination of hemoprotozoans in which 223 were recorded positive for hemoprotozoans. It's showed that the total prevalence is (57.47%) for the period of 04 year in sheep. Among the hemoprotozoan highest prevalence was recorded for Theileriosis which is (44.39%) followed by Anaplasmosis (29.14%), Mixed infection (19.73%) while lowest prevalence was recorded for babesiosis which is (6.72%) Table 2.

A total of 726 blood sample were analyzed for both sheep and goat. Out of the total blood sample (62.39%) total prevalence were recorded for all type of hemoprotozoa. In which highest prevalence was recorded for theleriosis 46.13%, followed by anaplasmosis 29.13%, mixed infection 17.66% while the lowest prevalence was recorded for babesiosis which is 7.06%. Table 3

SEASON WISE PREVALENCE OF HEAMOPROTOZOANS IN SHEEP

Seasonal wise overall prevalence was recorded highest in monsoon 48.69% followed by summer 34.34% and winter 16.95% in sheep. (Table 4). Same trends were recorded for theleriosis babesiosis, anaplasmosis and mixed infection in which highest prevalence percentage were recorded in moon soon followed by summer and winter season.

Season wise prevalence of heamoprotozoans in Goats

Season wise overall prevalence was recorded highest in monsoon 52.46% followed by summer 30.49% and winter 17.04% in goats. (Table 4). Same trends were recorded for theleriosis babesiosis, anaplasmosis and mixed infection

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Table 1: Prevalence of Different Heamoprotozoans in sheep in arid zone small ruminant station and the surroundingvillage of the station (Ghulam banda, shen dhand, babri banda and ghumbat) of the district kohat, (Pakistan).

	C	Cases Positive for Haemoprotozoans				
Year	Total Cases	Positive cases/Total prevalence	Theileriosis	Babesiosis	Anaplasmosis	Mixed
2017-18	56	40 (71.42%)	20(50%)	03 (7.5%)	13 (32.5%)	04 (10%)
2018-19	73	60(82.19%)	22 (36.66%)	08 (13.33%)	20(33.33%)	10(16.66%)
2019-20	113	70(61.94%)	30 (57.69%)	04 (7.69%)	23(44.23%)	13(25%)
2020-21	96	60(62.5%)	38 (63.33%)	02 (3.33%)	11 (18.33%)	09(15%)
	338	230 (68.04%)	110(47.82%)	17(7.39%)	67(29.13%)	36(15.65%)

Table 2: Prevalence of Different Heamoprotozoans in goats in arid zone small ruminant station and the surrounding village of the station (Ghulam banda, shen dhand, babri banda and ghumbat) of the district kohat, (Pakistan).

Cases Positive for Haemoprotozoans						
Year	Total Cases	Positive cases/Total prevalence	Theileriosis	Babesiosis	Anaplasmosis	Mixed
2017-18	63	47 (74.60)	15(31.91%)	04 (8.51%)	16 (34.04%)	12 (25.53%)
2018-19	96	49(51.04%)	26 (53.06%)	03(6.12%)	14(28.57%)	06(12.24%)
2019-20	88	62(70.45%)	29 (46.77%)	05 (8.06%)	20(32.25%)	08(12.90%)
2020-21	141	65(46.09%)	29 (44.61%)	03 (4.61%)	15 (23.07%)	18(27.69%)
	388	223 (57.47%)	99(44.39%)	15(6.72%)	65(29.14%)	44(19.73%)

Table 3: Overall Prevalence of Different Heamoprotozoans of sheep and goat in arid zone small ruminant station and the surrounding village of the station (Ghulam banda, shen dhand, babri banda and ghumbat) of the district kohat, (Pakistan).

Cases Positive for Haemoprotozoans						
Year	Total Cases	Positive cases/Total prevalence	Theileriosis	Babesiosis	Anaplasmosis	Mixed
2017-18	119	87 (73.10)	35(40.22%)	07(8.04%)	29(33.33%)	16(18.39%)
2018-19	169	109(64.49%)	48(44.03%)	11(10.09%)	34(31.19%)	16(14.67%)
2019-20	201	132(65.67%)	59(44.69%)	09(6.81%)	43(32.57%)	21(15.90%)
2020-21	237	125(52.74%)	67(53.6%)	05(04%)	26(20.8%)	27(21.6%)
	726	453(62.39%)	209(46.13%)	32(7.06%)	132(29.13%)	80(17.66%)

Table 4: Prevalence of Different Heamoprotozoans Season wise in sheep in arid zone small ruminant station and the surrounding village of the station (Ghulam banda, shen dhand, babri banda and ghumbat) of the district kohat, (Pakistan).

Season/weather	Positive cases/Total prevalence	Theileriosis	Babesiosis	Anaplasmosis	Mixed
Moon soon (JulyOctober)	112÷230×100=48.69%	57 (50.89%)	9(8.03%)	31(27.66%)	15(13.39%)
Summer (AprilJune)	79÷230×100=34.34%	36 (45.56%)	6(7.59%)	24(30.37%)	13(16.45%)
Winter (November March)	39÷230×100=16.95%	17 (43.58%)	2(5.12%)	12(30.76%)	8(20.51%)

in which highest prevalence percentage were recorded in moon soon followed by summer and winter season.

DISCUSSION

Result of the present study for the prevalence of haemoprotozoan parasites of small ruminants in arid zone kohat

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for different aspects such as prevalence in sheep, prevalence in goats, overall prevalence, prevalence among different season/weather and different small ruminants housing system are discussed below. **Table 5:** Prevalence of Different Heamoprotozoans Season wise in Goats in arid zone small ruminant station and the surrounding village of the station (Ghulam banda, shen dhand, babri banda and ghumbat) of the district kohat, (Pakistan).

Season/weather	Positive cases/Total prevalence	Theileriosis	Babesiosis	Anaplasmosis	Mixed
Moon soon (JulyOctober)	117÷223×100=52.46%	48(41.02%)	11(9.40%)	39(33.33%)	19(16.23%)
Summer (AprilJune)	68÷223×100=30.49%	32(47.05%)	4(5.88%)	17(25%)	15(22.05%)
Winter (November March)	38÷223×100=17.04%	19(50%)	0(0%)	9(23.68%)	10(26.31%)

In our study highest prevalence of hemoprotozoan were recorded in sheep (68.04%) as compared to goats (57.47%). Among the hemoprotozaon the theleriosis have the highest percentage as compared to the other protozoal disease. Highest prevalence of theileriosis has previously been reported in many studies (Durrani et al., 2011; Yin et al., 2007; Oura et al., 2004; El-Azazy et al., 2001). Our result is contrary with Jadhao et al. (2007) who studied the epidemiological pattern of haemoprotozoan infection of goats in the eastern zone of Maharashtra and found that only 14.72% goats positive for haemoprotozoal infection which was lower than our present findings. Similarly, Mohammed and Idoko (2013) reported 24.70% cases positive for hemoprotozoan in Nigerian goats which is much lower than our results. Difference in the results of present findings might be due to variations in geographical conditions, climatic variation, methodology adopted for the study and variation in the animal husbandry practices (Zangana and Naqid, 2011). The percentage of babesiosis in our study for sheep is 17% and for goat is 15% which is partially consistent with the results of (Mayzyad et al., 2002) who recorded 7.5% and 7.1% babesiosis and in goats and sheep, respectively. The percentage of anaplasmosis in our study for sheep is 67% and for goat is 65% which is contraindicated by (Djiba et al., 2013) who reported A. ovis very low in both sheep (4.32%) and goat (9.1%). Similarly (Li et al., 2020) reported the lower presence of anaplasma spp. The percentage of anaplasmosis in our study for sheep is 67% and for goat is 65% which is similar with that of the evaluations of (Dahmani et al., 2017) who also reported 69.7% prevalence in Nigeria similarly (Lee et al., 2018) reported 83.8% in Sudan and 89.1% in Uganda (Kasozi et al., 2021). in our results slightly a bit higher (67%) of anaplasmosis recorded in sheep as compared to goat (65%) which agree with the earlier studies recorded higher prevalence in Sheep compared with goats (Kasozi et al., 2021; Lee et al., 2018; Aouadi et al., 2017). Our results also not agree with the results of (Eisawi et al., 2020) who recorded higher prevalence of anaplasmosis in goats as compared to sheep in Sudan.

The highest prevalence of hemoprotozan was recorded in moon soon followed by summer and winter. Our results

are same with the earlier study of (Mohanta et al., 2011) who reported highest blood protozoa in the moon soon rainy spell (45.45%) followed by summer season (27.87%) and lowest in the winter (16.55%). In the present study high prevalence of hemoprotozons especially theileriosis observed it may be due to high abundance and occurrence of tick vector which transmit these hemoprotozoal disease and this high and humid weather is ideal for breeding and survival of ticks (Magona et al., 2011; Haque et al., 2010). This hot and humid environment favorable for the infestation of arthropod vectors which play pivot role in the transmission of hemoprotozoal diseases (Atif et al., 2012; Durrani, 2008; Sajid, 2007). Our results also in line with (Matioli et al., 1997) who described that monsoon rainy weather play most vital feature in the prevalence of Anaplasmosis and Babesiosis. Similarly (Zahid et al., 2005 and Muhammad et al., 1999) also reported that winter season having lower humidity and temperature are less favorable for the breeding, multiplication and growth of tick vectors due to which lower frequency of such diseases recorded. Our results are contraindicated by (Amin, 2015) who investigated prevalence in goat at Barisal, Bangladesh and resulted lowest prevalence in rainy weather (1.40%), highest in summer (3.82%) followed by winter (1.78%). The variation in the results may be due to considerable geographical seasonal variation and changes in the macroclimate of ticks which is critical and important for breeding of ticks.

High prevalence of hemoprotozoan were recorded in unorganized farm as compared to organized farm same results was reported by Patra et al. (2019) who reported highest prevalence in unorganized farm as compared to organized farm. Our results are inconsistent with Durrani et al. (2012) who recorded that herd size in small ruminants had no effect on the prevalence of hemoparasite in Khyber Pukhtoon Khwa and Punjab provinces in Pakistan.

CONCLUSION

Results of the present study concluded that the small ruminants of this part of kohat division is highly prevalent and endemic for hemoprotozon especially theleriosis, ana-

plasmosis and mixed bloold protozoal infection. The study also concluded that the sheep and goats rearing and rising in unorganized housing system are more prone to blood protozoal infection due to high load of carrier ticks which transmit the disease from one animal to another animal.

RECOMMENDATIONS

The present study recommend that technical staff of livestock department initiate the awareness program for surrounding local farmers and stakeholder of small ruminants on emergency basis. Technical staff should need to arrange the awareness program in the farm of field days and carrying out acaricidal spray for discouraging the tick life cycle, free veterinary camp for prevention and control of ticks, mass media campaign, radio talk, and encouraging close communication between farmers and Veterinary Research Institutes in the form of timely laboratory diagnosis of protozoan diseases. This study also recommended that the present study should also conduct in other zone of the country region having similar type of weather and climatic condition occur.

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CONFLICT OF INTEREST

The authors declare that they have no known competing interests.

NOVELTY STATEMENT

District kohat containing large number of small ruminants and no such work was done on the prevalence of hemoprotozoan in district kohat. This research work will direct livestock department for the prevention and control of hemoprotozan in district kohat.

AUTHORS CONTRIBUTION

Majid Ali: collected and analyze the blood sample, wrote the manuscript and corresponding with journal for publication.

Hafiz Abdul Majid: Review the data and statistical analysis.

Farman Ullah Khan: Laboratory analysis of blood sample

Tahir Khan = helped in the collection of blood sample. Muhammad Rashid Khan: Evaluate and improved the manuscript.

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