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ACTIVE SURVEILLANCE OF *BABESIA* INFECTION AND RISK FACTORS INVOLVED, IN SHEEP HOST, IN DISTRICT MULTAN, PAKISTAN

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ABSTRACT

This is the first cross sectional study in the region that involves the molecular detection of ovine *Babesia* from different farms located at different administrative towns in district Multan, Pakistan. Animal blood samples were screened for the presence of *Babesia* infection using PCR amplification assay. A total of 400 (69.8%) of 573 sheep investigated yielded 146 bp DNA and considered PCR positive for *Babesia* infection. The occurrences of *Babesia* spp. ranged from 57.9% to 83.6 0% from different towns of Multan. Statistically significant association ($p < 0.05$) of ovine babesiosis was found in younger animal compared to older ones. *Babesia* infection was detected significantly higher in Lohi breed (71.3%) compared to Kajli (54.6%) and Khadali (44.4%) breeds respectively. Tick infestation and season was found significant risk factors for the spread of ovine babesiosis. The high occurrence of *Babesia* infection in this study highlights the need for proper control and preventive measure of ovine babesiosis.

Keywords: *Babesia*, Sheep, PCR, Risk factors, Multan

INTRODUCTION

Livestock sector endured dominated by rural inhabitants to fulfill their milk and food requirement and source of cash income to uplift their socioeconomic conditions. The livestock added approximately 60.54% to the agriculture sector and 11.22 percent to gross

domestic product [1]. Sheep are the appropriate animals to exploit the meager vegetation available in arid environment through reseeded pastures [2]. Due to multi-facet utility for milk, meat, wool, manure and skin, sheep play an important role in Pakistan's agricultural economy. Sheep produced 39 thousand tons of milk, 45.1 thousand tons wool and 11.264 millions of skin during the fiscal year 2015-16 [1]. Livestock is at the risk due to tick borne diseases (TBDs) like babesiosis resulted in great economic losses around the world [3]. Optimal climatic conditions for accretion and agglomeration of ticks available in Pakistan being part of tropical areas resulted in plenty of tick diversity [4]. Tick-borne diseases (TTBDs) resulted in loss of 7000 million US$ annually around the globe, as 80 percent of cattle worldwide is at the risk of tick and tick borne diseases (TTBDs) [5].

Babesiosis is the third most important disease of sheep in Pakistan [6] caused by parasitic genus *Babesia,* that belongs to the order Piroplasmida of phylum Apicomplexa [7]. Ixodid ticks serve as vector for transmission of *Babesia* infection [8]. A total of six *Babesia* species caused babesiosis in sheep viz. *B. ovis*, *B. motasi*, *B. crassa*, *B. taylori*, *B. sp*. China and *B. foliate* [9]. *B. ovis* is deliberated the key etiologic agent of *Babesia* infection in sheep in Baluchistan and Punjab provinces of Pakistan [10]. The infected sheep characterized by fever, anemia, hemoglobinuria, jaundice, malaise, lethargy and anorexia during babesiosis. Alveolar edema and intrusion of neutrophils and macrophages in interstitial fluid, acute diffused proliferative glomerulitis, immobility in glomerular capillaries and severe necrosis during ovine babesiosis as histopathological effects has been reported previously [11]. The information regarding ovine babesiosis in Pakistan is very limited. Previously, *Babesia* piroplasms had been detected mostly through microscopy [6, 12, 13, 14]. Livestock sector demands sober control and management of animal health, which is possible through early finding of disease and its therapy [9]. The development of an expeditious, exact and sensitive diagnostic method for pathogen identification is crucial for treating and controlling or even eradicating infectious disease. Due microscopic limitations, the current study was aimed: (i) to assess the prevalence of *Babesia* infection in sheep through PCR and (ii) to find risk factors that involved in spread of *Babesia* infection in and around Multan district, Pakistan.

MATERIALS AND METHODS

Study sites:

Multan is located on the southern side of Province Punjab, Pakistan and comprising of six towns shown in Figure (1) namely Shah Rukn-e-Alam, Sher Shah, Bund Bosan, Mousa Pak, Shuja-Abad and Jalalpur and referred as Strata or Clusters. Within these clusters (primary units) selected herds were secondary units from which the third or final units "sheep" were selected. A multistage cluster sampling method was used to find incidence of babesiosis in the field avoiding a specific farm study [15].

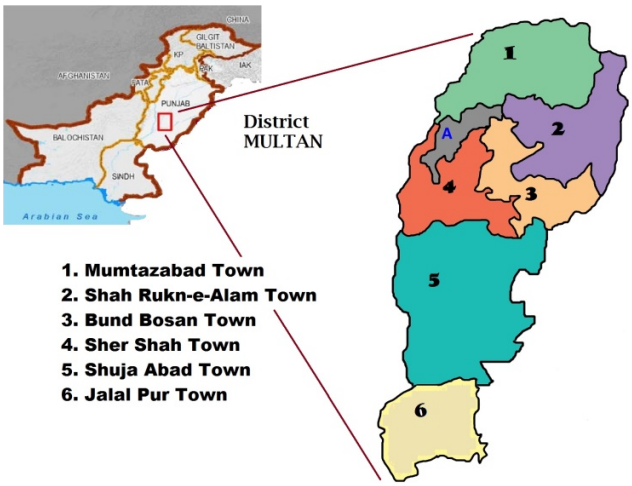

Figure 1: Study Area: District Multan and its administrative towns

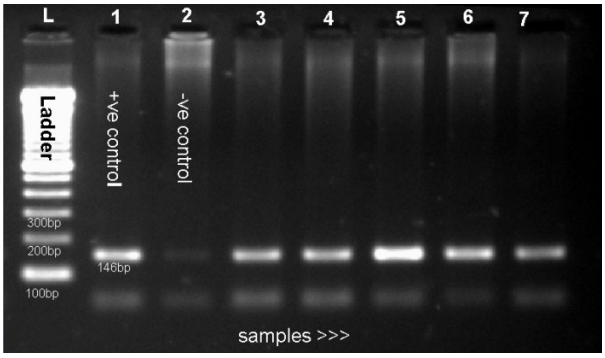

Figure 2: PCR products for *Babesia* spp. using genus specific primers resulting in 146bp amplicons

Statistical Analysis

For statistical analysis, animals were divided into 3 age groups: up to 1year old, 1- 2 years old and more than 2 years old. To assess the seasonal impact samples were divided into Pre-Summer (Feb-Mar) and Post-Summer (Sep-Oct). The prevalence of ovine babesiosis and the various parameters i.e. sex, breed and age, tick burden, area (town-wise location) and season was evaluated by using

the Fisher's exact test (for 2x2 tables) and Chi-square test.

RESULTS AND DISCUSSION

Out of 573 animal blood samples tested, 400 (69.8%) yielded 146 bp DNA fragment were positive for *Babesia* infection. Higher prevalence was detected in Shahruknealam town (83.3%) and lower in Shuja abad town (57.8%) as indicated in table (1).

There was significant ($p < 0.05$) association between babesiosis and different sampling sites. Based on PCR findings in this study, it appears that ovine babesiosis is widespread in our sheep, with as high as 83.8% *Babesia* detection rate being reported in the studied area. During earlier studies conducted in Pakistan, the prevalence of ovine babesiosis was investigated through presence of symptoms and microscopic examination in infected animals. Microscopic screening method revealed 9.7%, 13% and 23.5% from Pakistan during earlier studies [6, 12, 13]. With the use of molecular techniques 50% prevalence in sheep from southern Punjab [18] and 29% prevalence was reported at Livestock Experiment Station, Okara [19]. During current survey PCR detected 69.8% prevalence which was higher so far within and outside Pakistan. The results of higher prevalence are in agreement with findings from Egypt [20], Iran [21, 22] and Iraq [23] where up to 57% prevalence of *Babesia* in sheep has been reported. But contrary to present study, lower prevalence as low as just 5% was studied in Iran [21, 24, 25], Tunisia [26] and Turkey [27]. The difference of infection rate of babesiosis endorsed due to geo-climatic conditions, availability of tick vectors and genetic resistance against babesiosis in the studied sheep breeds. *Babesia* infection is found higher in tropical and sub-tropical regions [22] due to favorable geo-climatic conditions for tick growth. Chronic infections of babesiosis with low parasitaemia are common [28] which are microscopically undetectable and serve as reservoir for infection in the herds, since animals that are not clinically ill may continue to infect with tick vector [17].

A higher prevalence of babesiosis was noted in males (70.7%) as compared to females (64.1%) investigated in this study while the association was non-significant between gender and *Babesia* infection. Several findings [17, 22, 26, 29, 30, 31] support current results of non- significant correlation between ovine babesiosis and gender of animal. But contradictory to present study, Shahabuddin *et al*. [12] and Iqbal *et al*. [18] reported significant association between gender and *Babesia* infection might endorsed due to anatomical and physiological

especially hormonal differences resulted in variations.

Table 1: Area wise PCR amplification results of sheep from Multan, Southern Punjab, Pakistan during 2013

District	Area	Total sample	Positive (%)	Negative (%)
Multan	Shahruknealam	102	85 (83.3)	17 (16.7)
	Bund Bosan	84	49 (58.3)	35 9(41.7)
	Shershah	89	65 (73)	24 (27)
	Musapak Shaheed	112	87 (77.7)	25 (22.3)
	Shuja abad	95	55 (57.9)	40 (42.1)
	Jalalpur	91	59 (64.8)	32 (35.2)
Total		573	400 (69.8)	173 (30.2)

Chi-Sq = 25.297, DF = 5, P-Value = 0.000

Table 2: Association between occurrences of *Babesia* parasites identified by PCR in sheep and the studied parameters describing animal characters in Multan, Southern Punjab, Pakistan during 2013

Parameters		No. of samples	Piroplasms Positive (%)	Piroplasms Negative (%)	P* Value
Sex	Male	78	50 (64.1)	28 (35.9)	
	Female	495	350 (70.7)	145 (29.3)	0.23[a]
Age	≤ 1 year	178	110 (61.8)	68 (38.2)	
	≤ 2 year	198	123 (62.1)	75 (37.9)	
	≥ 2year	197	142 (72)	55 (28)	0.05[b*]
Breed	Lohi	533	380 (71.3)	153 (28.9)	
	Kajli	22	12 (54.6)	10 (45.4)	0.00 [b*]
	Khadali	18	8 (44.4)	10 (55.6)	0.01[b*]
Ticks	Present	400	300 (75)	100 (25)	*0.01[b]
	Absent	173	100 (57.8)	73 (42.2)	
Season	Pre-summer	312	189 (60.6)	123 (39.4)	
	Post-summer	261	211(80.8)	50 (19.2)	0.00 [a*]

a = Fisher's exact test b = Chi square test;

Babesia infection found significantly higher in older animals (72%) than younger animals (61.1%) in the studied population of sheep and correlation was significant. The results are in agreement with Fakhar *et al*. [22]; Rjeibi *et al*., [26] and Razmi et al. [29] who reported higher prevalence in older animals compared to youngerones. The findings are contrary to Iqbal et al. [18] and Shahzad *et al* [19] who reported higher prevalence of *Babesia* infection younger than older animals, because of low immunity level in young age compared with older sheep. According to Sevinc *et al*., [32] age of the animal is one of the most important factors that affect the susceptibility to babesiosis. Lambs of smaller age mostly impervious to severe infections due to passive immunity gained through colostrum feeding and non-specific natural resistance. Lambs infested with infected tick during this period become resistant to re-infection, but later infestation when passive immunity has lost infection rate might be higher. It takes time to develop active immunity that's why animals older

than one year have strong immunity as compared to young ones.

The collected data showed the significant correlation between babesiosis and tick infestation on sheep. The threat of haemoprotozoan was found to be greater after higher tick burden on domestic animals [33]. The results revealed significant association of babesiosis and tick infestation in sheep and tick positive animals more infected than animals without ticks. The results are in accordance to Iqbal et al. [18] and Aktas *et al*. [34] who reported higher infection rate in animals infested with ticks. These findings established the role of ticks as vector for transmission of babesiosis. The higher prevalence of babesiosis in tick infested animals endorsed that piroplasmosis is related with tick activity and tick growth [35]. No breeds were found free from *Babesia* infection, however Lohi breed was more infected (71.3%) followed by Kajli (54.6%) and lower in Khadali (44.4%) during present study. Results were justified with reference to difference of genetic resistance of local breeds to piroplasms.

Babesia infection was reported significantly higher ($p < 0.05$) in post summer (88.8%) than pre summer (60.5%) during current study. The results confirm the findings of Fakhar *et al*., [22] and Razmi *et al*., [29] who reported higher prevalence of *Babesia* infection during August and September respectively which corresponds to the most vivid season of the adult vector ticks. Researchers [36, 37] and [38] had reported strong correlation between prevalence of ovine babesiosis and tick activity. Ahmad *et al.,* [39] reported a significant variation in *Babesia* infection during different seasons and recorded higher prevalence during summer and autumn.

CONCLUSIONS

As end result of this study it represents the frequency of *Babesia* infection in sheep was found 400 (69.8%) through PCR amplification in Multan District, Pakistan. Female and older animals were more infected with *Babesia* infection while Lohi breed was more prone to *Babesia* infection. The consequence of the study also administrates that ovine babesiosis is an imperative tick borne transmitted disease and further investigation is required for understanding the epidemiology of the ovine babesiosis.

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