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PREVALENCE AND ASSOCIATED RISK FACTORS OF BOVINE BABESIOSIS IN LAHORE, PAKISTAN

Sadaqat Ali[1, 2], Muhammad Ijaz[1*], Arslan Ahmed[1], Muhammad Umair Aziz[1], Muhammad Naveed[1], Muhammad Umar Javed[1], Yasir Nawab[1], Nauman Zaheer Ghumman[1]and Awais Ghaffar[1]

[1]Department of Clinical Medicine and Surgery, University of Veterinary and Animal Sciences, Lahore 54000, Punjab, Pakistan.
[2]University College of Veterinary and Animal Sciences, The Islamia University of Bahawalpur, Bahawalpur 61300, Punjab, Pakistan.

Corresponding author: mijaz@uvas.edu.pk

ABSTRACT

Babesiosis is a tick-borne infectious disease caused by intra-erythrocytic protozoan parasites of the genus *Babesia.* The present study was conducted to investigate the prevalence and related associated risk factors of bovine babesiosis in district Lahore, Pakistan. A total of 1258 animals (n = 532 buffaloes; n = 726 cattle) were sampled through random sampling technique and analyzed for the detection of inclusion bodies resembling babesiosis through thin smear microscopy. Risk factors regarding breed, specie, age, month of the year, gender, and season were statistically analyzed using chi-square test on SPSS to find the association of different risk factors with the occurrence of this protozoan pathogen. The study has revealed an overall 34.02% prevalence of babesiosis in bovines in district Lahore. The infection rate was statistically insignificant (P>0.05) in cattle's (34.57%) compared to buffaloes (33.27%). The females are at more risk of having babesiosis as compared to males in cattle (OR=01.24, CI=0.82-1.89) as well as buffaloes (OR=01.32, CI=0.81-2.14). The study concludes that babesiosis is prevalent in study district and adult animas and summer months were found significantly associated with the occurrence of this tick-borne disease. These study findings will aid in establishment of better strategies for prevention and control of disease

Keywords: Bovine Babesiosis, Risk factors, Prevalence, Microscopy

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INTRODUCTION

Animal's health and production are badly affected due to tick borne diseases. Ectoparasites not only suck the blood and other body tissue fluids but also can transfer different diseases to the hosts (Ramzan et al. 2008).

Babesiosis is caused by a protozoan parasite belonging to genus *Babesia*. Red blood cells of wild and domestic animals are parasitized by this malaria-like protozoan. This disease was first discovered in cattle by a Romanian scientist Victor Babes in 1888, who described the relation of intra-erythrocytic bodies with red urine or hemoglobinuria (Babes 1888). Now, the disease is well recognized in cattle, horses and dogs, which has gained increasing consideration as an emerging zoonotic threat (Homer et al. 2000; Siddique et al. 2020). According to an estimate, over fifty percent of the total world's cattle population is likely to be infected by babesiosis (Bock et al. 2004). Various species of babesia are involved in causing disease in different geographical areas of the world mainly *B. bovis* and *B. bigemina* especially in tropical areas of the world. These affects the economics of countries located in tropical and sub-tropical parts of the world, where livestock farmers getting milk, meat and required protein sources of high nourishing quality from cattle farming (Vial and Gorenflot 2006; Pupin et al. 2019). The disease is generally clinically characterized by the extensive erythrocyte destruction leading to anemia, icterus, hemoglobinuria and finally death. *Babesia* does not require an exoerythrocytic phase instead it directly invades the RBCs and starts multiplying by binary fission and results in red blood cell destruction and eventually hemoglobinuria, however, saliva of ticks is compulsory for completion of life cycle of this parasite (Gohil et al. 2013). The distribution of different species of *Babesia* depends upon the type of *Ixodid* ticks present in that area because these ticks are responsible for disease dissemination. *Rhipicephalus annulatus* and *Rhipicephalus microplus* are the most frequent source of dispersion for both *B. bovis* and *B. bigemina* and are mainly found in subtropical and tropical regions of the world including Asia. Contrary to this, *Ixodes ricinus* is responsible for the

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transmission of *B. divergens* in Europe (Zintl et al. 2003; Bock et al. 2004; Chauvin et al. 2009). Microscopy detection methods are still the cheapest and fastest methods used to identify *Babesia* parasites (Mosqueda et al. 2012). Several species of ticks are reported in domestic animals in Pakistan (Ali et al. 2019; Batool et al. 2019). Babesiosis has previously been detected by using direct smear microscopy (Ahmad et al. 2014), and PCR in bovines in Pakistan (Siddique et al. 2020).

The present study was conducted to investigate the prevalence of babesiosis in bovines in district Lahore, Pakistan. The association of associated risk factors with the occurrence of this protozoan pathogens was also calculated.

MATERIALS AND METHODS

***Study Rationale*:** The sampling was executed from district Lahore which is located on eastern bank of the river Ravi at longitude 74°21'2.5" E while latitude 31°33'28.8' N'. The area coincides with district Nankana Sahib on west, district Kasur in south, India on east while district Sheikhupura on the west and north. The study area has different climatic changes throughout the year; a monsoon, dust storms and extremely hot summer and dry winter (World Weather Online 2012; Time and Date 2012). The documented values of pressure, temperatures and humidity of individual month (for year 2012) are given in Table 1 for district Lahore (Fig. 1).

Table 1: Pressure, temperatures and humidity of individual month of year 2012 in District Lahore

Month	Pressure (Hg)			Humidity (%)			Temperature (°F)			Precipitation (mm)
	High	Low	Average	High	Low	Average	High	Low	Average	
Jan	30.21	29.74	30.02	100	19	66	72	34	53	3.58
Feb	30.15	29.68	29.97	94	18	55	79	36	57	0.46
Mar	30.09	29.53	29.87	94	16	48	95	48	71	2.37
Apr	30.01	29.59	29.77	94	15	47	100	61	81	14.61
May	29.80	29.33	29.64	57	12	30	115	68	92	3.48
Jun	29.68	27.88	29.44	89	14	38	115	73	97	1.14
Jul	29.62	29.27	29.45	94	10	62	115	73	92	10.33
Aug	29.92	29.30	29.57	100	19	74	106	72	87	52.51
Sep	29.95	29.47	29.70	100	19	71	99	72	85	65.36
Oct	30.09	29.65	29.90	100	36	62	97	59	78	2.61
Nov	30.15	29.59	29.96	100	34	65	86	48	68	0.03
Dec	31.90	29.53	30.01	100	26	73	82	32	56	0.64

Source: World Weather Online, 2012; Time and Date, 2012.

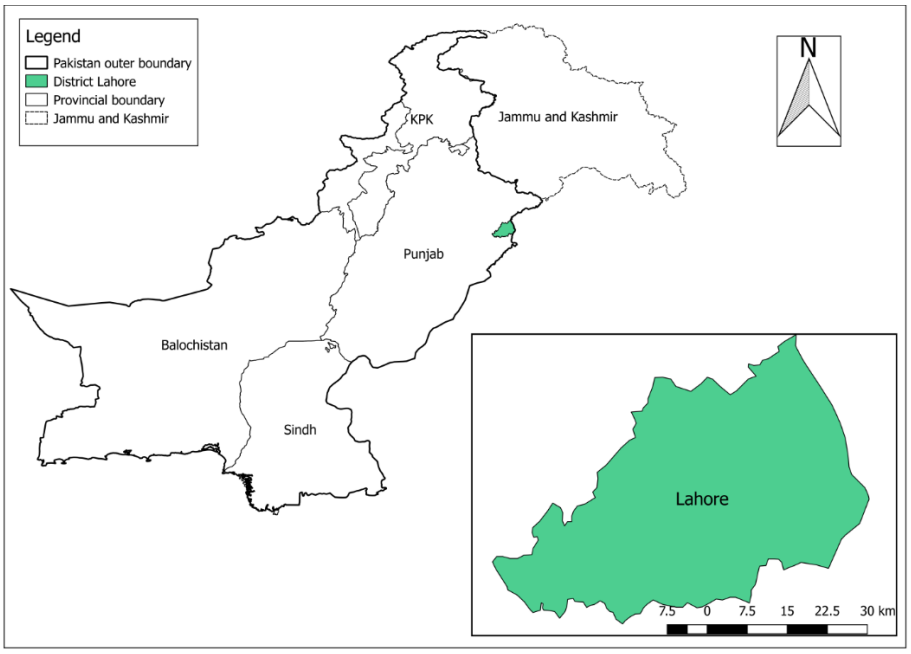

Fig. 1: Map of the study district.

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The study was conducted in district Lahore during the year 2012 (from January to December) in order to determine the prevalence of babesiosis and association of hypothesized risk factors with the occurrence of this piroplasmid pathogen. A total of 1258 bovines (n = 726 cattle; n = 532 buffaloes) were included in this study by random sampling strategy from various public and private sector veterinary hospitals and livestock farms. The sampling was conducted irrespective of age, sex and breeds of study animals. There were no specific inclusion or exclusion criteria for sampling. Samples were collected from both apparently diseased and healthy animals

***Sampling*:** Thin blood smears (in triplets) were prepared by ear tip puncture, labeled and air dried at spot. The samples were then transported to Medicine laboratory, Department of Clinical medicine and Surgery, University of Veterinary & Animal Sciences (UVAS), Lahore. The blood smears were then fixed with alcohol (100% methyl alcohol) for 3 minutes; afterward stained with 10% Giemsa's stain for almost 45 minutes and rinsed with distilled water. The blood smears on slides were then examined under binocular microscope at oil immersion objective (100×). The samples having intra-erythrocytic inclusion bodies resembling *Babesia* (Fig. 2) were then declared positive (Farooqi et al. 2017).

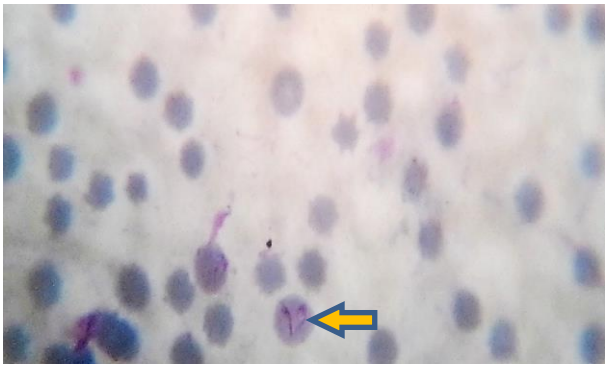

Fig. 2: Picture shows *Babesia* like inclusion bodies inside the erythrocytes by Giemsa's staining.

***Risk Factors*:** In order to find out the association of hypothesized risk factors with the occurrence of babesiosis, a questionnaire was designed. Different variables like age of animals [(calf having age less than 1 year), young (1 to 3 years), adult (3 to5 years), and old (more than 5 years)], sex of animals, specie and breed of animals, month of sampling (January to December), season of sampling (Ahmed et al. 2019): [summer (June to August), autumn (September to November), winter (December to February) and spring (March to May)] were recorded in questionnaire.

***Statistical Analysis*:** The data regarding risk factors were analyzed by descriptive statistics and Chi-square test ($\chi 2$) using IBM statistical product and service solutions (SPSS) version20.0 software. The odd ratios were then calculated by univariable analysis (Farooqi et al. 2017). The variables showing *p*-value less than 0.05 were considered significantly associated with babesiosis.

RESULTS

The results of the study revealed an overall 34.02% prevalence of babesiosis in bovines in the study area. The infection rate was higher in cattle (34.57%) compared to buffaloes (33.27%). Although non-significantly (P>0.05) associated, but the odds of having infection was 1.06 % (CI=0.84-1.34) more in cattle as compared to buffaloes (Table 2).

Table 2: Prevalence of babesiosis in bovines in district Lahore

Variable	Total	Positive	Negative	Prevalence (%)	Odd Ratios (OR)	95% CI of OR	P-value
Buffalo	532	177	355	33.27	Ref	-	-
Cattle	726	251	475	34.57	1.0598	0.84-1.34	0.6301
Total	1258	428	830	34.02			

In cattle's, adult stocks were most widely affected group (OR=1.45, CI=0.93-2.27), followed by young stock (OR=1.41, CI=0.89-2.21), olds (OR=0.76, CI=0.48 to 1.20) and calves respectively (Table 3). While in buffaloes

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infection rate was more in adult stocks (OR=1.48, CI=0.85-2.60) followed by young stock (OR=1.48, CI=0.85-2.55), old (OR=0.93, CI=0.54-1.60) and calves respectively (Table 4). Friesian cattle/cow was most susceptible breed in cattle's (OR=1.17, CI=0.66-2.07), followed by cross-bred (OR=1.14, CI=0.69-1.91), non-descript, Sahiwal (OR=0.98, CI=0.58-1.68) and Cholistani (OR=0.95, CI=0.54-1.69) respectively (Table 3). While in buffaloes, non-descript breed was affected more as compared to Nili-Ravi (OR=0.99, CI=0.69-1.43) (Table 4). Cattles were affected more in summer (OR=13.16, CI=07.84-22.10), followed by autumn (OR=03.53, CI=2.33-5.35), spring (OR=13.16, CI=07.84-22.10) and winter (OR=02.58, CI=01.39 to 04.45) (Table 3). While in buffaloes, most the disease was more prevalent in autumn (OR=14.15, CI=6.5752-30.4678), followed by summer (OR=06.59, CI=3.46-12.56), winter (OR=01.18, CI=0.57-02.45) and spring respectively (Table 4). In cattle's infection rate was found to be highest during July (OR=26.83, CI=7.38-97.54) (Table 3) while in buffalos' infection was more in September (OR=48.75, CI=12.58-188.83) (Table 4). The prevalence was more in females as compared to males in cattle (OR=01.24, CI=0.82-1.89) (Table 3) as well as buffaloes (OR=01.32, CI=0.81-2.14) (Table 4).

Table 3: Summary of risk factors of babesiosis in Cattle

Variable	Category	Total	Positive	Negative	Prevalence (%)	Odd Ratios (OR)	95% CI of OR	P value
Month	Jan	43	5	38	11.63	2.01	0.45-08.99	0.3573
	Feb	45	10	35	22.22	4.38	1.12-17.12	0.0337
	Mar	71	12	59	16.9	3.11	0.83-11.70	0.0919
	Apr	62	11	51	17.74	3.30	0.86-12.59	0.0796
	May	63	14	49	22.22	4.38	1.18-16.24	0.0271
	Jun	71	42	29	59.15	22.20	6.29-78.30	<0.0001
	Jul	55	35	20	63.63	26.83	7.38-97.54	<0.0001
	Aug	83	42	41	50.6	15.70	4.52-54.53	<0.0001
	Sep	93	40	53	43.01	11.57	3.35-39.90	<0.0001
	Oct	37	21	16	56.76	20.12	5.28-76.61	<0.0001
	Nov	54	16	38	29.63	6.45	1.74-23.82	0.0051
	Dec	49	3	46	6.12	Ref	-	-
Season	Summer	209	133	76	63.63	13.16	7.84-22.10	0.0001
	Autumn	184	61	123	33.15	3.53	2.33-05.35	0.0001
	Winter	137	34	103	24.81	2.48	1.39-04.45	0.0022
	Spring	196	23	173	11.73	Ref	-	-
Breed	Sahiwal	173	57	116	32.95	0.98	0.58-01.68	0.9492
	Cholistani	124	40	84	32.26	0.95	0.54-01.69	0.8673
	Cross-bred	217	79	138	36.41	1.14	0.69-01.91	0.6045
	Holstein Friesian	119	44	75	36.97	1.17	0.66-02.07	0.5823
	ND	93	31	62	33.33	Ref	-	-
Sex	Female	605	214	391	35.37	1.24	0.82-01.89	0.3121
	Male	121	37	84	30.58	Ref	-	-
Age	Calf	208	55	153	26.44	0.76	0.48-01.20	0.24
	Young	173	69	104	39.88	1.41	0.89-02.21	0.1404
	Adult	189	77	112	40.74	1.45	0.93-02.27	0.0964
	Old	156	50	106	32.05	Ref	-	-

DISCUSSION

The study has revealed an overall 34.02% prevalence of babesiosis in bovines. However, the low prevalence of babesiosis have also been reported in the country in bovines by various authors; 17.23% (Siddique et al. 2020),

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Table 4: Summary of risk factors of babesiosis in Buffaloes

Variable	Category	Total	Positive	Negative	Prevalence (%)	Odd Ratios (OR)	95% CI of OR	P value
Month	Jan	41	4	37	9.76	1.22	0.28 - 5.20	0.7917
	Feb	41	3	38	7.32	0.89	0.19 - 4.22	0.8814
	Mar	50	7	43	14	1.83	0.50 - 6.70	0.3608
	Apr	55	6	49	10.91	1.38	0.36 - 5.20	0.6365
	May	80	20	60	25	3.75	1.99 - 11.73	0.0232
	Jun	51	31	20	60.78	17.43	5.42 - 56.01	<0.0001
	Jul	32	23	9	71.88	28.75	7.98 - 103.45	<0.0001
	Aug	34	21	13	61.76	18.17	5.28 - 62.46	<0.0001
	Sep	32	26	6	81.25	48.75	12.58 - 188.8	<0.0001
	Oct	37	20	17	54.05	13.23	3.94 -44.37	<0.0001
	Nov	30	12	18	40	7.50	2.13 -26.35	0.0017
	Dec	49	4	45	8.16	Ref	-	-
Season	Summer	197	95	102	48.22	06.59	3.46-12.56	<0.0001
	Autumn	69	46	23	66.66	14.15	6.57-30.47	<0.0001
	Winter	161	23	138	14.29	1.18	0.57-02.45	0.6574
	Spring	105	13	92	12.38	Ref	-	-
Breed	Nili Ravi	253	84	169	33.20	0.99	0.69-1.43	0.97
	ND	279	93	186	33.33	Ref	-	-
Sex	Female	437	150	287	34.32	1.32	0.81-2.14	0.27
	Male	95	27	68	28.42	Ref	-	-
Age	Calf	168	47	121	27.98	0.93	0.54-1.60	0.80
	Young	139	53	86	38.13	1.48	0.85-2.55	0.160
	Adult	123	47	76	38.21	1.48	0.85-2.60	0.167
	Old	102	30	72	29.41	Ref	-	-

9.67% (Rashid et al. 2010), 7.2% (Niazi et al. 2010), 6.57% (Atif et al. 2012), 2.85% (Khan et al. 2004), 2.80 % (Afridi and Ahmad 2005) and 2.5% (Zahid et al. 2005). Higher prevalence has been reported by various studies; from 70 100% (Bell et al. 2004; Oliveira et al. 2005). The variation in prevalence might be due to difference in sampling strategy, different geographical locations, seasons of sampling, different management practices, diagnostic technique used and accuracy in diagnostic techniques used. The prevalence was slightly higher in cattle's (34.57%) compared to buffaloes (33.27%) in this study. Similar findings were also recorded in various other studies (Vahora et al. 2012; Li et al. 2014; Siddique et al. 2020). This might be due to difference in inclusion criteria for sampling and vector load on the host species.

Higher occurrence of disease in females as compared to males has also been reported previously (Alim et al. 2012; Siddique et al. 2020). Few studies have reported more prevalence in males (Atif et al. 2012; Farooqi et al. 2017). The higher prevalence in females in this study could be due to the fact that females may suffer more stress as compared to males, specifically during milking due to hormonal imbalance and during pregnancy (Kabir et al. 2011), ultimately, they are at more risk of having infection. Moreover, there were comparatively more females included in this study as compared to males, which can be a cause of more prevalence. Adult animals were most widely affected group, followed by young stock, olds, and calves. These Findings were in line with Urquhart et al. (1996) and Annetta et al. (2005), who have reported an inverse age resistance of the disease where adult showed more susceptibility than calves. This might be due to rapid immune responses to primary infection by the calves through a complex immune mechanism (Annetta et al. 2005). Alim et al. (2012) also recorded increased babesiosis with the increase of age and highest prevalence was observed in adult crossbred cattle. Contrary to this, Amorim et al. (2014) has identified that the calves were more susceptible to *Babesia*. Although breed is insignificant factor in both species, Friesian was most susceptible breed, followed by cross bred, non-descript, Cholistani and Sahiwal in cattle, while in buffaloes, non-descript was most affected breed as compared to Nili Ravi. The variation in prevalence in different breeds might be due to the fact that due to repeated exposures of parasitic diseases, there is enhanced immune response in native breeds as compared to the imported breeds (Siddiki et al. 2010).

Season was also found significant risk factor affecting the prevalence of babesiosis. Babesiosis was most commonly present in summer, followed by autumn, winter, and spring among cattle, but in buffaloes, it was most prevalent in autumn, followed by summer, winter, and spring. Other studies have also reported more prevalence of babesiosis in summer and autumn (Siddique et al. 2020). There is high temperature and humidity in summer season

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which supports the propagation of ticks (Randolph 2004). In cattle's July was found most prevalent month while in buffaloes infection was more in September. Infection rate was found more in summer days as compared to the other days which might be due to the favorable conditions for the propagation of ticks, which are the vectors for the transmission of this disease (Sayin et al. 2003; Qayyum et al. 2010).

Conclusion: The study has revealed 34.02% prevalence of babesiosis in bovines in district Lahore. The infection rate was slightly higher in cattle's (34.57%) as compared to buffaloes (33.27%). The prevalence was more in females as compared to males. The infection was found higher in summer months in both cattle and buffaloes which might be due to the favorable conditions for the propagation of ticks which are the vectors for the transmission of this disease. So, by controlling the vectors the problem can effectively be addressed. These study findings will aid in establishment of better strategies for prevention and control of disease. Further research focusing the molecular epidemiology and sequencing of babesiosis should be conducted to implement better control measure against this tick-borne malaise.

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Contribution of Authors: MI designed the project. SA did sampling from the study area and processed the samples. AA, NZG, MUA and MN compiled the data into excel sheet and analyzed the data statistically. MUJ, YN and AG wrote the manuscript. MI, SA and AG reviewed the manuscript critically.

Conflict of Interest: Authors declare no conflict of interest.

ORCID

Sadaqat Ali https://orcid.org/0000-0001-6671-854X
Muhammad Ijaz https://orcid.org/0000-0002-0628-7773
Arslan Ahmed https://orcid.org/0000-0002-6589-8208
Muhammad Umair Aziz https://orcid.org/0000-0002-0075-713X
Muhammad Naveed https://orcid.org/0000-0002-5433-1839
Muhammmad Umar Javed https://orcid.org/0000-0002-2883-0642
Yasir Nawab https://orcid.org/0000-0002-7399-3723
Nauman Zaheer Ghumman https://orcid.org/0000-0003-2499-5965
Awais Ghaffar https://orcid.org/0000-0002-1878-2480

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