PREVALENCE OF TICK-BORNE DISEASES IN PUNJAB (PAKISTAN) AND HEMATOLOGICAL PROFILE OF *ANAPLASMA MARGINALE* INFECTION IN INDIGENOUS AND CROSSBRED CATTLE

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ABSTRACT: The study was designed to investigate the prevalence and distribution of tick-borne diseases (TBDs) and hematological parameters associated with *Anaplasma marginale* infection in cattle. A total of 1050 blood samples were randomly collected from selected small holders and private livestock farms using multistage cluster random sampling technique. Microscopic examination of the Giemsa stained blood smears revealed an overall prevalence of blood parasites as 21.14%. *Anaplasma marginale* was the most prevalent hemoparasite (5.81%) followed by *Theileria* spp. (5.14%) and *Babesia bigemina* (4.76%), respectively. Chi square analysis indicated a significant association (P<0.05) between districts as well as between indigenous and crossbred cattle. However, a non-significant association between different age groups, seasons, sex and farm sizes was revealed. Haemolytic anaemia was the key clinical manifestation of *Anaplasma marginale* infection. It has been concluded that tick-borne pathogens were prevalent in selected districts of Punjab, Pakistan.

Key words: Cattle, *Anaplasma*, *Babesia*, *Theileria,* Punjab.

INTRODUCTION

Tick-borne diseases (TBDs) are mostly prevalent in tropical and subtropical regions of the world including Pakistan (Jongejan and Uilenberg, 2004; Khan *et al*., 2004). Anaplasmosis is the most prevalent hemo-rickettsial disease of cattle in Pakistan (Khan *et al*., 2004). Earlier reports has mentioned the prevalence of *Anaplasma marginale* ranging from 4-75.5%, *Babesia bigemina* 1.75-7.2%, *Theileria annulata* 0.7-24% (Khan *et al*., 2004; Afridi *et al*., 2005 Rajput *et al*., 2005; Ahmad and Hashmi, 2007; Niazi *et al*., 2008). The prevalence varies from region to region and various factors determine the occurrence of the TBDs including age, sex, breed, tick density, season, geographical area and management (Kivaria, 2006; Magona *et al*., 2011). Hemolytic anaemia is the major clinical manifestation of *Anaplasma marginale* infection in cattle (Hofmann-Lehmann *et al*., 2004). Earlier reports cited above lack distribution of TBDs on the basis of age, sex, breed, season and herd size which may be of significance in understanding the epidemiology of TBDs. Therefore, the present study was aimed at investigating the prevalence of TBDs in Sargodha, Khushab and Rawalpindi districts of the Punjab.

MATERIALS AND METHODS

A survey on bovine TBDs was conducted at Sargodha, Khushab and Rawalpindi districts of the Punjab, Pakistan from September, 2009 to August, 2010. A total of 1050 blood samples were collected from randomly selected small holders (n=90) and private livestock farms (n=12) using multistage cluster random sampling technique (Thrusfield, 2005). All the union councils in each district were included in the sampling frame. A total of 30 union councils, 34 cattle farms (30 small holders and 4 livestock farms) and 350 animals were selected as primary, secondary and elementary sampling units from each district. Sampling unit was indigenous and crossbred cattle of both the sexes. Animals were sampled in different age groups i.e. <1 year, 1-2 year, >2-4 year and >4 years. Prevalence was estimated using formula: $P= d/n \times 100$; where P= Prevalence, d= No. of animals found positive, n= Total no. of animals sampled (Thrusfield, 1995).

Blood samples were collected aseptically in a sterilized syringe from jugular vein for the detection of *Anaplasma* and *Theileria* species. Whereas, blood from ear vein was used for babesiosis. Thin blood smears were prepared as described by Houwen (2000). The blood parasites were identified as described by OIE (2004); OIE (2008a) and OIE (2008b).

Hematological study: A total of 90 blood samples

(Sahiwal n=45 and crossbred n=45) were collected from *Anaplasma marginale* infected cattle and ten non-infected cattle (Sahiwal n=5 and crossbred cattle n=5) as control. The level of parasitaemia was determined by Giemsa stained blood smear. The hematological changes were studied at different levels of parasitaemia <7%, >7-15% and >15%. Total erythrocyte count (TEC), total leukocyte count (TLC), haemoglobin (Hb), packed cell volume, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) were evaluated using haematology analyzer (Sysmex, PocH 100, USA) in infected and non-infected cattle.

The prevalence of TBDs was analyzed by chi square. Analysis of Variance was used to evaluate significant difference among hematological parameters at different levels of parasitaemia and pair-wise comparison of hematological parameters were performed using Tukey test. The hematological parameters between Sahiwal and crossbred cattle were analyzed using student's *t*-test. Statistical Package for Social Services (SPSS) version 13.0 was used for statistical analysis. A p-value <0.05 was considered statistically significant.

RESULTS

Out of 1050 samples, 222 were declared positive for tick-borne pathogens with an over all prevalence of 21.14%. *Anaplasma marginale* was the most prevalent (5.81%; 61/1050) haemoparasite followed in order by *Theileria* spp. (5.14%; 54/1050) and *Babesia bigemina* (4.76%; 50/1050). The percentage of tick-borne pathogens having mix infection (5.43%; 57/1050) were: *A. marginale* with *Babesia bigemina* (1.62%; 17/1050), *A. marginale* with *Theileria* spp. (2.67%; 28/1050) and *B. bigemina* with *Theileria* spp. (1.14%; 12/1050). Highest prevalence of TBDs was recorded in Sargodha (24.86%; 87/350) and lowest in Rawalpindi district (17.14%; 60/350). The prevalence of *Theileria* spp. infection was highest in Sargodha district while *Anaplasma marginale* was highest in Khushab followed by Rawalpindi district (Table 1). There was a significant association (*P*<0.05) between the prevalence of tick-transmitted diseases among Sargodha, Khushab and Rawalpindi districts.

The highest prevalence of *Anaplasma marginale* (9.52%; 20/210) and *Babesia bigemina* (6.19%; 13/210) was observed at 2-4 years of age while *Theileria* spp. infection (8.08%, 16/198) at 1-2 years of age (Table 1). Chi-square value indicated a non-significant association (χ^2= 2.32, df= 6, *P*>0.05) among different age groups. The prevalence of *Anaplasma marginale* and *Theileria* spp. infection was higher in female while *Babesia bigemina* were higher among male (Table 1). Statistical analysis revealed non-significant association (χ^2=1.623, df= 2 *P*>0.05) among male and female animals. The prevalence of tick-transmitted diseases was higher in crossbred animals as compared to indigenous cattle (Table 1). A significant association (χ^2=6.910, df= 2, *P*<0.05) on the prevalence of TBDs between indigenous and crossbred cattle was found. Similarly non-significant association (χ^2= 0.319, df= 2, *P*>0.05) was observed between small holders and livestock farms on the prevalence of tick transmitted diseases. However, higher prevalence was recorded in small holders (105/450, 23.33%) as compared to livestock farms (60/600, 10.00%).

The highest prevalence of cattle TBDs was recorded in summer season in all study districts. Seasonal prevalence of TBDs had non-significant association in all study districts (Sargodha, χ^2= 6.824, df= 6, *P*>0.05; Khushab, χ^2= 4.814, df= 6, *P*>0.05; Rawalpindi, χ^2= 2.033, df= 6, *P*>0.05).

Table 1. Age, sex and breed wise prevalence (%) of *Anaplasma marginale*, *Babesia bigemina* and *Theileria* spp. using Giemsa stained blood smear among cattle in Sargodha, Khushab and Rawalpindi districts, Punjab, Pakistan from September, 2009 to August, 2010.

Factor Variable		***A. marginale* (P/E*)**	***B. bigemina* (P/E)**	***Theileria* spp. (P/E)**
Age < 1		3.14% (10/318)	3.14% (10/318)	3.77% (12/318)
1-2		7.58% (15/198)	6.06% (12/198)	8.08% (16/198)
>2-4		9.52% (20/210)	6.19% (13/210)	5.71% (12/210)
>4		4.94% (16/324)	4.63% (15/324)	4.32% (14/324)
Total		5.81% (61/1050)	4.76% (50/1050)	5.14% (54/1050)
Sex	Male	4.85% (13/268)	5.60% (15/268)	4.10% (11/268)
	Female	6.14% (48/782)	4.48% (35/782)	5.50% (43/782)
Total		5.81% (61/1050)	4.76% (50/1050)	5.14% (54/1050)
Breed	Indigenous	2.10% (11/525)	3.62% (19/525)	3.81% (20/525)
Crossbred**		9.52% (50/525)	5.90% (31/525)	6.48% (34/525)
Total		5.81% (61/1050	4.76% (50/1050)	5.14% (54/1050)

Age: χ^2 value= 2.32, df= 6, *P*>0.05; **Sex:** χ^2 value= 1.623, df= 2, *p*-value= >0.05; **Breed:** χ^2 value= 6.910, df= 2, p-value <0.05; *P= No. positive, *E= No. examined; **(Indigenous x Holstein Friesian)

Table 2. Hematological parameters (mean ± SE) in *Anaplasma marginale* infected Sahiwal and crossbred cattle associated with different levels of parasitaemia.

Parasitaemia	No. of animals	WBC $X10^3/\mu L$	RBC $X10^6/\mu L$	PCV %	Hb g/dL	MCV(fL)	MCH (pg)	MCHC (g/dL)
1-7%	21	6.83b±0.14	5.73b±0.10	26.7b±0.27	9.83b±0.08	58.98a±0.05	21.13a±0.09	36.76a±0.13
>7-15%	18	6.55b±0.12	4.91c±0.07	24.9bc±0.71	7.94c±0.08	58.41b±0.06	19.40b±0.14	34.32b±0.32
>15%	6	6.34b±0.12	4.47c±0.16	20.6c±1.66	7.03d±0.14	58.28b±0.04	17.50c±0.46	30.43c±0.97
Crossbred cattle								
1-7%	16	6.56 b ±0.16	4.47b ±0.11	25.4b ±0.29	8.95 a ±0.09	59.39 a ±0.06	23.18a±0.10	37.97±0.22a
>7-15%	22	6.43 b ±0.10	3.51c ±0.06	21.0c ±0.43	7.23 b ±0.12	59.29 b ±0.0	22.10b±0.09	35.62b±0.32
>15%	7	6.25 b ±0.09	3.38 c ±0.10	19.7c ±0.77	6.94 b ±0.34	59.22 b±0.03	20.17c±0.42	31.78b±0.93
Healthy control	5	7.43 a ±0.17	6.50 a ±0.08	32.4a ±1.32	10.02 a ±0.85	49.50 c±0.18	16.53d±0.08	33.41b±0.07

Means with letters (a, b, c) denote statistically significant difference (ANOVA; $P<0.05$).

All the Sahiwal cattle showed a significant difference ($P<0.01$) in hematological parameters (WBCs, $P<0.01$; RBC, $P<0.001$; PCV, $P<0.001$; Hb, $P<0.001$; MCV, $P<0.001$; MCH, $P<0.001$; MCHC, $P<0.001$) at different levels of parasitaemia. With the increase in parasitaemia and decrease in (RBCs, PCV, Hb, MCV, MCH and MCHC) blood parameters was observed. The values of the blood parameters in clinically healthy and *Anaplasma marginale* infected crossbred and Sahiwal cattle are presented in Table 2. All the crossbred cattle also showed a significant difference ($P<0.001$) in hematological parameters (WBCs, $P<0.001$; RBC, $P<0.001$; PCV, $P<0.001$; Hb, $P<0.001$; MCV, $P<0.001$; MCH, $P<0.001$; MCHC, $P<0.001$) at different levels of parasitaemia (1-7%, >7-15 and >15%).

Microscopic examination of the blood smears showed nucleated RBCs, basophilic stippling and anisocytosis at 1-7% parasitaemia. No significant ($P>0.05$) difference was recorded between hematological values of Sahiwal and crossbred cattle for WBC, Hb, MCV and MCHC. Conversely, significant difference was observed for RBCs, PCV and MCH values between Sahiwal and crossbred cattle.

DISCUSSION

The epidemiology of TBDs has not been fully explored, despite the fact that they cause major constraint on livestock production and health in Pakistan (Khan *et al*., 2004). District-wise prevalence of tick-transmitted diseases was found significant. The major part of the study districts has a distinct agro-ecological zone that supports the significant association on the prevalence of TBDs among districts. Contrasting reports on the prevalence of TBDs have been published. The prevalence of *Anaplasma marginale* infection in cattle from Peshawar and Hyderabad regions have been recorded as 4.2% and 22%, respectively (Rajput *et al*., 2005; Afridi *et al.,* 2005). On the contrary, higher prevalence of *Anaplasma marginale* 75.7% has been reported at two government livestock farms located at Islamabad and Attock district as compared to the present study (Khan *et al.,* 2004).

Khan *et al*. (2004) and Afridi (2005) have reported comparatively lower (1.42% and 0.70%, respectively) prevalence of *Theileria* spp. in cattle on microscopic examination of blood smears. Whereas, Zahid *et al.* (2005) mentioned higher (24%) infection of *Theileria* species in exotic Holstein Friesian cattle. Previous reports lack distribution of TBDs on the basis of age, sex, breed, season and herd size. Moreover, there was difference of area and cattle breeds under study. The prevalence varies from region to region, host, management and agro-ecological and geo-climatic factors influence the prevalence of ticks and TBDs (Kivaria, 2006).

Zahid *et al.* (2005) reported the prevalence of *Babesia* spp. as 5% at government Livestock Experiment Station, Kasur district. Similarly, Ahmad and Hashmi (2007) have reported the overall occurrence of *Babesia bigemina* as 6.6% in cattle from Malakand Agency. While Afridi *et al*. (2005) has reported a lower prevalence (1.75%) of *Babesia bigemina* from Peshawar and adjoining areas as compared to present study.

The significant relationship of different breeds on the prevalence of TBDs was found. The findings of Khan *et al*. (2004) are in accord with our work who stated that crossbred cattle (19.4%) are more susceptible to TBDs than indigenous Red Sindhi (17%) and Dhanni (14%) breeds. The European breeds are more susceptible to TBDs due to higher infestation of ticks (Rodostits *et al*., 2007).

The highest prevalence of TBDs was recorded in summer and lowest in winter. The highest abundance of the ticks has been reported in July (Sajid, 2007) whereas; *Hyalomma* spp. of ticks were the most abundant in June (Durrani, 2008). Durrani associated the prevalence of Theileriosis with tick abundance in June (Durrani, 2008). Ahmad and Hashmi (2007) have reported the highest prevalence of ticks and *Babesia bigemina* infection in cattle during the month of August in Malakand. This area lies in northern high land cooler region of Pakistan.

Summer season starts earlier in Punjab as compared to the northern parts of the country in which tick season generally starts late. The prevalence of *Anaplasma marginale* in winter in the absence of vector ticks suggests mechanical transmission.

Extravascular haemolytic anaemia is the key sign of *Anaplasma marginale* infection in cattle (Kuttler, 1984; Ajayi *et al*., 1978). Anaemia results due to phagocytosis of parasitized erythrocytes in spleen and bone marrow (Jain, 1993). Usually intravascular and extravascular haemolysis takes place simultaneously (Riond *et al*., 2007). Along with the destruction of parasitized erythrocytes the destruction of non-parasitized erythrocytes also takes place due to immune mediated autolysis. Antibodies were produced against *Anaplasma marginale* infected RBCs as well as against its non-infected red blood cells. The level of parasitaemia and the percentage of anaemia do not have relationship during anaplasmosis (Schroeder and Ristic, 1968).

Increased MCV and decrease of MCHC is usually the indication of regenerative anaemia. The MCV was found higher in infected as compared to healthy control in both Sahiwal and crossbred cattle. Microscopic examination of the blood smears showed nucleated RBCs, basophilic stippling and anisocytosis at 1-7% parasitaemia. On account of that MCV had increased in *Anaplasma* infected cattle. The decrease of MCHC and increase of MCV was noticed as compared to healthy controls in both breeds. This classify the anaemia as hypochromic and macrocytic. The increase in MCV is usually the indication of regenerative anaemia (Riond *et al*., 2007). After rapid destruction of RBCs by phagocytosis the immature RBCs are released from bone marrow due to increase demand. The immature RBCs are larger in size than mature red blood cells explain the reason for increased MCV. It has been concluded that TBDs are distributed in selected districts of Punjab, Pakistan. There is a need for further investigations using advanced serological and molecular tools for the identification of tick-borne pathogens.

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