

SEROPREVALENCE OF *ANAPLASMA MARGINALE* INFECTION AMONG CATTLE FROM THREE DISTRICTS OF THE NORTHERN PUNJAB, PAKISTAN

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ABSTRACT

The study was designed to investigate the seroprevalence of *Anaplasma marginale* among cattle from Sargodha, Khushab and Rawalpindi districts of the northern Punjab, Pakistan. A total of 1050 samples were collected from selected small holders and private livestock farms using multistage cluster random sampling technique. The overall seroprevalence of *Anaplasma marginale* infection was 31.05 percent % using competitive enzyme-linked immunosorbent assay (cELISA). The highest seroprevalence was recorded in Sargodha (37.14%) district followed by Khushab (31.43%) and Rawalpindi (24.57 %) districts. A significant relationship was found among different age groups and breed. The seroprevalence was significantly higher in small holders than private livestock farms in all study districts. It is concluded that small holder's crossbred cattle of more than four years of age from Sargodha district are more susceptible to *Anaplasma marginale* infection in summer season.

Key words: Seroprevalence, *Anaplasma marginale*, cattle, northern Punjab.

INTRODUCTION

Anaplasmosis is one of the important globally distributed tick-borne disease of cattle with great economic impact (Minjauw and Mcleod, 2003). Bovine anaplasmosis (BA) is caused by obligate intraerythrocytic rickettsia of the order *Rickettsiales*, family *Anaplasmataceae*, genus *Anaplasma* (Dumler *et al.*, 2001) characterized by fever, weight loss, decreased milk production, pale mucous membranes, severe anaemia, jaundice, brownish urine, hyper-excitability abortion and mortality without hemoglobinemia and hemoglobinuria during acute phase of the infection (Richey and Palmer, 1990).

Serological tests commonly used for the serodiagnosis of *Anaplasma marginale* including agglutination test, indirect fluorescent antibody test, complement fixation and indirect ELISA. There were problems of sensitivity, reproducibility, interpretation and non-specific reactions associated with these tests (OIE, 2004; OIE, 2012). Competitive ELISA has obvious advantage because of greater sensitivity 96% and specificity 95% (Urdaz-Rodriguez *et al.*, 2009).

There exist very scanty information if ever available on the serological survey of *Anaplasma marginale* infection in cattle using competitive enzyme linked immunosorbent assay from northern, Punjab. Most of the earlier reports from Pakistan were based on the examination of stained blood smears (Atif *et al.*, 2012).

In Pakistan, the prevalence of *Anaplasma marginale* has been recorded as 7.36-75.71 percent using microscopic examination of blood smears (Khan *et al.*, 2004, Afridi *et al.*, 2005; Rajput *et al.*, 2005). This technique usually fails to detect chronically infected carriers with low levels of parasitaemia (Kocan *et al.*, 2010). The identification of carriers are important for epidemiological standpoint as well as for planning disease prevention and control strategies. Therefore, the present study was designed to investigate the prevalence of *Anaplasma marginale* infection from Sargodha, Khushab and Rawalpindi districts of the Punjab, Pakistan.

MATERIALS AND METHODS

Study area: Epidemiological studies were conducted in Sargodha, Khushab and Rawalpindi districts of the Punjab, Pakistan. Sargodha district has average temperature ranging from 25-49°C in summer and 5-23°C in winter and annual rainfall of 526 millimeter. Khushab is the driest and hottest district. The temperature ranges from 25-48°C in summer and 19-29°C in winter with average annual precipitation of 521 millimeter. Rawalpindi district is categorized as arid high rainfall zone (PARC, 2012). The average temperature ranges from 23.5-35.5°C in summer and 10-17.5°C in winter with annual average rainfall of 1364 millimeter (GOP, 2012).

Sampling strategy: A serological survey on the prevalence of *Anaplasma marginale* was conducted at Sargodha, Khushab and Rawalpindi districts of the Punjab, Pakistan from indigenous and crossbred cattle during 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) and serum was separated. 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 tertiary sampling units from each district. Animals were sampled in different age groups i.e. < 1 year, 1-2 year, > 2-4 year and > 4 years. The criteria for the selection of small holders and private livestock farms were: a) small holder having 1-10 cattle; (b) Livestock farm having 50 cattle; (c) Distance between small holder farms 5 kilometer; (d) Distance between livestock farms ≥ 10 kilometer.

Serological screening: Antibodies against *A. marginale* in serum were detected by MSP-5 competitive enzyme linked immunosorbent assay (cELISA) using commercially available Anaplasma Antibody Test Kit, cELISA (VMRD Inc., Pullman, WA, USA) validated previously by Urdaz-Rodriguez et al. (2009). The test was performed according to the manufacturer's instruction (VMRD Inc., Pullman, WA, USA).

An ELISA reader (Statfax[®] 2100 Microplate Reader, Awareness Technologies, Inc.) was used to measure the optical density at 620 nm wavelength. A cutoff of 30% inhibition was used to differentiate between positive and negative samples. Serum samples with 30% inhibition were considered positive and samples with <30% inhibition were considered negative. The percent inhibition was calculated using formula:

$$\text{Inhibition percentage} = 100 \frac{[\text{Sample optical density}]}{[\text{Mean negative control optical density}]} \times 100$$

Prevalence was estimated using formula: $P = \frac{d}{n} \times 100$; where P= Prevalence, d= No. of animals found positive, n= Total no. of animals sampled (Thrusfield, 1995).

Statistical analysis: The data was statistically analyzed by applying Chi square test using Statistical Package for Social Services (SPSS) version 13.0. A *p*-value <0.05 was considered statistically significant.

RESULTS

Out of 1050 samples, 326 (31.05%) were found positive for *Anaplasma marginale*. Highest seroprevalence was recorded in Sargodha district (37.14%) followed by Khushab (31.43%) and Rawalpindi (24.57%) districts (Table 1). There was significant

difference in seroprevalence of *Anaplasma marginale* among districts. Irrespective of the district under study, a significant ($P < 0.001$) association among different age groups was recorded. The highest seroprevalence of *Anaplasma marginale* was found in >4 years age as compared to other age cohorts i.e. <1, 1-2 and >2-4 years. Though seroprevalence was higher in females compared to males (Table 1); but statistically it was non-significant ($P > 0.05$). Regardless of the district under study, seroprevalence of *Anaplasma marginale* was significant between breeds. The seroprevalence was higher in crossbred as compared to indigenous cattle. Similarly, significant association was observed on the seroprevalence of *Anaplasma marginale* infection among small holders in all districts whereas, seroprevalence had non-significant association at livestock farms (Table 1). The highest (39.60%) seroprevalence of *Anaplasma marginale* was recorded in summer in all study districts. Seasonal prevalence was found significant in Sargodha and Khushab districts, while non-significant association was revealed in Rawalpindi district.

Table 1. Seroprevalence of *Anaplasma marginale* among cattle in Sargodha, Khushab and Rawalpindi districts of the Punjab, Pakistan from September, 2009 to August, 2010.

Variables	Levels	n/N	Prevalence (%)
Age	<1 year	47/318	14.78*
	1-2 years	42/198	21.21*
	>2-4 years	80/210	38.10*
	>4 years	157/324	48.46*
Sex	Male	76/268	28.36
	Female	250/782	31.97
Breed	Indigenous	137/525	26.10*
	Crossbred	189/525	36.00*
Season	Autumn	61/174	35.06
	Winter	81/351	23.08
	Spring	45/174	25.86
	Summer	139/351	39.60*
Farm size	Small holder*	157/450	34.89*
	Cattle farm	169/600	28.17
District	Sargodha	130/350	37.14*
	Khushab	110/350	31.43*
	Rawalpindi	86/350	24.57*

n= no. of tick infested cattle; N= total no of cattle examine; * significant association, chi square *p*-value <0.05; df= degree of freedom; **Age:** ($\chi^2 = 98.99$, df= 3, $P < 0.001$); **Sex:** ($\chi^2 = 1.215$, df= 1, $P > 0.05$); **Breed:** ($\chi^2 = 12.02$, df= 1, $P < 0.001$); **Seasonal prevalence: Sargodha** ($\chi^2 = 12.95$, df= 3, $P < 0.01$); **Khushab** ($\chi^2 = 8.82$, df= 3, $P < 0.05$); **Rawalpindi** ($\chi^2 = 5.763$, df= 3, $P > 0.05$); **Small holder** ($\chi^2 = 7.76$, df= 2, $P < 0.05$); **Livestock farms** ($\chi^2 = 5.552$, df= 2, $P > 0.05$); **District:** ($\chi^2 = 12.95$, df= 2, $P < 0.05$);

DISCUSSION

It was hard to find any previous reference regarding sero-prevalence of *Anaplasma marginale* infection in northern Punjab, Pakistan. There were variations in the distribution of *A. marginale* infection in different geographical regions. The seroprevalence was statistically significant among all study districts. Major parts of study districts have distinct agro-ecological zone (PARC, 2012), justify the significant difference in seroprevalence among study districts. Moderate climate of Sargodha district favours the growth and multiplication of vector ticks. Moreover, higher prevalence of *R. (Boophilus) microplus* and stall feeding practices could have possibly accounted for higher prevalence of *Anaplasma marginale* infection at Sargodha. Marufu *et al.* (2010) have recorded the seroprevalence as 26percent in semi-arid, sweet and sour rangeland of South Africa using competitive inhibition ELISA. The seroprevalence is quite comparable with Rawalpindi and Khushab districts having arid environment.

The seroprevalence recorded in the present study was 31.05%, pointing the region is rather endemically unstable. Endemic stability most likely occurs in regions where serum antibodies prevail in 70% of animal population (Perry and Young, 1995; Peter *et al.*, 1997). The competitive ELISA was used for the detection of serum antibodies as recommended by World Animal Health Organization for the serodiagnosis of anaplasmosis in cattle (OIE, 2004). Competitive ELISA based on major surface protein-5 has obvious advantage over other serological tests because of higher sensitivity 96% and specificity 95% for anaplasmosis (Urdaz-Rodriguez *et al.*, 2009). The cELISA uses a 19-kDa antigen based on recombinant major surface protein (MSP5) which is highly conserved among *Anaplasma* species (Knowles *et al.*, 1996).

Agreement was found on the age-wise prevalence of *Anaplasma marginale* with Swai *et al.* (2005) and Urdaz-Rodriguez *et al.* (2009). The significant relationship of different breeds on the prevalence of *Anaplasma marginale* was found. Similarly, Khan *et al.* (2004) mentioned higher prevalence of tick-borne disease in cross bred cattle (19.4%) than indigenous Red Sindhi (17%) and Dhanni (14%) breeds. Lower seroprevalence in indigenous cattle indicate inherent resistance to ticks which results in lower *Anaplasma marginale* infection (Swai *et al.*, 2007). Tick resistance trait most likely contributed lower *Anaplasma marginale* infection. The European breeds are more susceptible to tick-borne diseases due to higher infestation of ticks (Bock *et al.*, 1997).

Higher prevalence of *Anaplasma marginale* in small holders (34.89%) endorse the findings of Urdaz-Rodriguez *et al.* (2009) who also reported an increasing

trend in seropositivity in small holders than medium and large livestock farms. Hugh-Jones *et al.* (1998) found no association between herd size and seroprevalence while Perez *et al.* (1994) documented that seroprevalence increased with increasing herd size in dairy animals. Swai *et al.* (2005) considered that higher seroprevalence in small holders might be associated with specific age group. The association of herd size with *Anaplasma marginale* seropositivity was found non-significant ($P>0.05$) in the present study. Poor management, lack of tick control practices and inadequate economic sustainability of poor resource small holder farmers for the implementation of proper management and animal health practices contributed the higher seroprevalence (Swai *et al.*, 2005; Gralen, 2009). It is concluded that Sargodha district's small holder crossbred cattle of more than four years of age are more susceptible to *Anaplasma marginale* infection in summer.

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