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Prevalence and Risk Factors of Anaplasmosis in Cattle and Buffalo Populations of District Khanewal, Punjab, Pakistan

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Abstract: A cross-sectional survey was conducted in 836 cattle {*Bos (B.) indicus* and *B. taurus x B. indicus*} and 700 buffaloes (*B. bubalis bubulus*) (n=1536) of district Khanewal, Punjab, Pakistan, in order to determine the epizootiology of anaplasmosis through conventional optical microscopy of Giemsa's stained blood films. With an overall prevalence of 4.17% (64/1536); the distribution of anaplasmosis was higher in buffaloes than cattle, calves than adults, females than males. Anaplasmosis in selected animal population was found statistically associated with the breed, season, animal keeping, housing system and hygienic management. The collected information provides the first report of anaplasmosis in the study population of the region. The data will not only be helpful for the dairy farmers to modulate farming practices but also for the policy and decision makers to control the nuisance in the livestock population of the district. However, use of modern molecular tools is suggested for differential diagnosis of *Anaplasma* from other haemoprotozoa like *Babesia* and *Theileria* on a wider geographical spectrum.

Key words: Anaplasmosis · Prevalence · Associated Factors · Bovine · Khanewal · Punjab · Pakistan

INTRODUCTION

Anaplasmosis is an economically important disease caused by the members of genus *Anaplasma* (Rickettsiales: Anaplasmataceae). In cattle and buffaloes, this disease is caused by *Anaplasma (A.) marginale* and *A. centrale* [1]; later less pathogenic than former [2]. The disease is endemic in the tropics and subtropics; however, reports are available from temperate regions too [3,4]. Transmission of *Anaplasma* is possible biologically through genera of *Boophilus, Rhipicephalus* and *Hyalomma* vectors [5] and mechanically through contaminated needles and biting flies of the family Tabanidae. Mammals, birds or ticks may also act as natural reservoirs for anaplasmosis if persistently infected by the disease [1]. Prevalence of anaplasmosis is found higher in hot and humid weather associated with the abundance of vector ticks [6,7].

After tick bites, anaplasma enter the erythrocyte for their replication which leads to busting of R.B.Cs (Haemolysis) [1]. Clinical signs and symptoms of anaplasmosis includes: icterus, fever, weakness, anaemia, jaundice without haemoglobinemia and haemoglobinuria, decreased milk production, weight loss, abortion, hyperexitability due to cerebral anoxia and sudden death [8]. Animals can show clinical signs and symptoms 20 days post-infection with R.B.C. infectivity of 15% [8]. Prevalence of anaplasmosis has been reported in cattle or buffaloes or from both worldwide including Costa Rica (59.6%) [9], Italy (50%) [3], Iran (50%) [10], China (32.35%) [11] and Morocco (21.9%) [12] by using optical microscopy, PCR or ELISA as diagnostic technique, while from Pakistan only few studies have been reported from Attock (75.92%), Islamabad (66.66%) [13], Sargodha (37.14%), Khushab (31.43%) and Rawalpindi (24.57 %) [14]. In continuation with the existing knowledge, the present study was planned with the objectives to determine, a) the prevalence of anaplasmosis in cattle and buffaloes of district Khanewal and b) an association of different host, agent and environment related risk factors

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with the disease distribution. The data will not only be helpful for the dairy farmers to modulate farming practices but also for the policy and decision makers to control the nuisance in the livestock population of the district in specific and country in general.

MATERIALS AND METHODS

Study Area: Study area (District Khanewal) lies at 30°18'0 N 71° 55'0 E with an altitude of 128 metres in Punjab province of Pakistan and has an area of 3259 Km^2. Average temperature ranges from 13.8°C to 29.8°C; while average annual precipitation is 10.5mm. It includes four tehsils, namely Khanewal, Mian Channu, Kabirwala and Jahanian. There are 100 union councils in this district. Buffalo and cattle population of the district are 566219 and 424036 heads respectively [15].

Selection of Animals: Simple random sampling was used for the selection of study animals. A total of 1536 animals (836 cattle and 700 buffaloes) were calculated using win episcope (Version 2.0) and screened during a period of one year (May 2011 to April 2012). Ninety six farms each having at least 10 animals and placed nonetheless 5 Km^2 away from other screened farms, were selected for screening anaplasmosis by using map grid method [16]. Sample fraction in present study was calculated as 0.0016% (1536/990255). Figure 1 shows the physical boundaries of district Khanewal where the black dots indicate the villages selected for the survey.

Epidemiological Investigation: A questionnaire containing open-ended and closed ended (Dichotomous or multiple choice) questions was designed to procure the required information [16] and it was refined through conducting a pilot survey before the commencement of the surveillance. Host related determinants included: species (Buffalo and cattle), breeds (Sahiwal and cross bred in cattle; Kundi and Nili Ravi in buffalo), age (Adult and young) and sex (Female and male). Specie (*A. marginale* and *A. centrale*) was the only agent related determinant. Environment related determinants included: season (Summer, autumn, winter and spring), animal keeping (Open and tethered), housing (Closed, semi-closed and open), floor pattern (Cemented, partially-cemented and un-cemented), water management (Tap and canal) and hygienic measures {ranked from 1 to 10; categorized as good (8-10), poor (4-7) and very poor (1-3)}.

Blood Collection and Examination: Blood samples (3-5ml) were collected from the jugular veins of the animals in EDTA containing vacutainers and transported to the Department of Parasitology, University of Agriculture

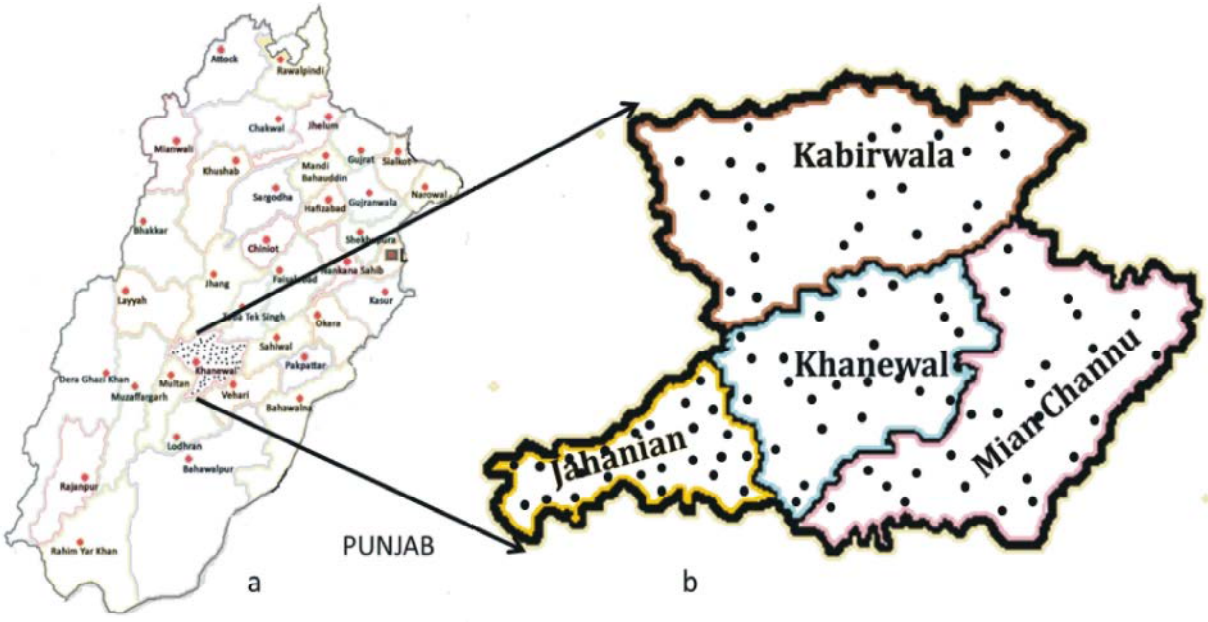

Fig. 1: (a) Physical map of Punjab where target area (district Khanewal) is highlighted by dots (b) map of district Khanewal with its tehsils including Khanewal, Mian Channu, Kabirwala and Jahanian and in each tehsil black dots depict the screened farms.

Faisalabad in ice bags for microscopic examination following the method of Adam, Paul and Zaman [17]. Briefly, a thin blood smear was prepared from each blood sample, air dried and fixed in methanol for 2-3 minutes. Staining was done in 5% Giemsa's stain and rinsing was performed in two changes of distilled water buffered to pH 7.2. Examination of the smears was performed at 100X magnification with compound microscope by searching at least 50 fields per slide. The parasites were identified as described by OIE [18].

Statistical Analysis: Epidemiology of bovine anaplasmosis was determined by using multiple logistic regression and odd's ratio (OR) at 95% confidence level [19]. Pair-wise comparisons were carried out at by keeping cattle, Sahiwal, Nili Ravi, adults, males, winter season, tehsil Khanewal, open animals, open housing system, good hygienic measures, cemented floor pattern and tap watering system in group 2 of analysis. All statistical analysis were conducted using SAS software package [20].

RESULTS

Overall prevalence of anaplasmosis in study population was found 4.17% (64/1536) with 4.07% (34/836) in cattle and 4.29% (30/700) in buffaloes. Comparative distribution of anaplasmosis in cattle and buffaloes did

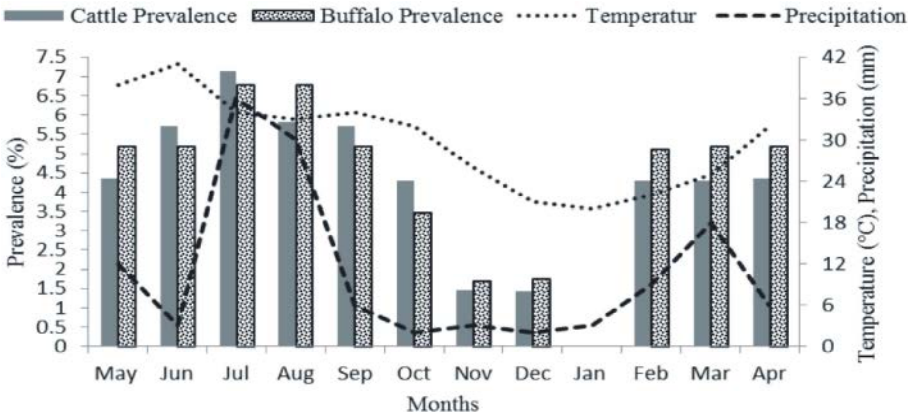

Fig. 2: Monthly distribution of anaplasmosis in cattle and buffaloes of district Khanewal, Punjab Pakistan. Dotted lines indicate association of the disease with temperature (°C) and precipitation (mm) on the secondary Y-axis.

Table 1: Prevalence of anaplasmosis and risk factors in cattle population of district Khanewal, Punjab, Pakistan

					Confidence interval 95%			
Variables	Levels	Animals Screened	*Anaplasma* Positive	Prevalence (%)	Lower limit	Upper limit	Odds Ratio	P-value
Breed	Cross Bred	432	26	5.99	60.16	88.43	3.04	0.004
	Sahiwal	404	8	1.98	11.57	39.84	-	-
Age	Young Stock	390	22	5.64	47.72	79.27	2.10	0.044
	Adults	446	12	2.69	20.73	52.28	-	-
Sex	Female	439	24	5.47	53.81	83.99	2.17	0.044
	Male	397	10	2.52	16.01	46.19	-	-
Specie	*A. marginale*	836	24	2.87	53.81	83.99	2.17	0.044
	A. centrale	836	10	1.20	16.01	46.19	-	-
Season	Summer	209	12	5.74	20.73	52.28	6.00	0.009
	Autumn	209	11	5.26	18.33	49.27	5.50	0.016
	Spring	209	9	4.31	13.75	43.05	4.50	0.048
	Winter	209	2	0.96	01.00	18.10	-	-
Area (Tehsils)	Jahanian	213	10	4.69	16.01	46.19	1.24	0.724
	Kabirwala	205	8	3.90	11.57	39.84	1.03	0.900
	Mian Channu	207	8	3.86	11.57	39.84	1.02	0.900
	Khanewal	211	8	3.79	11.57	39.84	-	-

Table 1: Prevalence of anaplasmosis and risk factors in cattle population of district Khanewal, Punjab, Pakistan

					Confidence interval 95%			
		Animals	*Anaplasma*					
Variables	Levels	Screened	Positive	Prevalence (%)	Lower limit	Upper limit	Odds Ratio	P-value
Animal Keeping	Tethered	438	23	5.25	50.73	81.67	2.52	0.018
	Open	398	11	2.76	18.33	49.27	-	-
Housing System	Closed	279	15	5.38	28.25	60.95	3.15	0.017
	Semi-closed	294	14	4.76	25.69	58.11	2.79	0.048
	Open	293	5	1.71	5.59	29.63	-	-
Hygienic Measures	Very Poor	293	17	5.80	33.54	66.46	4.68	0.008
	Poor	301	14	4.65	25.69	58.11	3.75	0.027
	Good	242	3	1.24	02.29	22.16	-	-
Floor Pattern	Un-Cemented	292	16	5.48	30.87	63.73	3.64	0.016
	Partially-Cemented	278	14	5.04	25.69	58.11	3.35	0.023
	Cemented	266	4	1.50	03.82	25.98	-	-
Watering System	Canal	429	19	4.43	39.05	71.75	1.20	0.666
	Tape	407	15	3.69	28.25	60.95	-	-

Table 2: Epizootiology of anaplasmosis in buffaloes of district Khanewal, Punjab, Pakistan

					Confidence interval 95%			
		Animals	*Anaplasma*					
Variables	Levels	Screened	Positive	Prevalence (%)	Lower limit	Upper limit	Odds Ratio	P-value
Breed	Kundi	268	16	5.97	35.59	70.46	1.84	0.106
	Nili Ravi	432	14	3.24	29.54	64.41	-	-
Age	Young Stock	326	20	6.13	48.51	81.67	2.29	0.032
	Adults	374	10	2.67	18.33	51.42	-	-
Sex	Female	398	23	5.78	59.21	89.18	2.49	0.030
	Male	302	7	2.32	10.82	40.79	-	-
Specie	*A. marginale*	700	20	2.86	48.51	81.67	2.29	0.032
	A. centrale	700	10	1.43	18.33	51.42	-	-
Season	Summer	175	10	5.71	18.33	51.42	5.00	0.027
	Autumn	175	9	5.14	15.73	47.97	4.50	0.047
	Spring	175	9	5.14	15.73	47.97	4.50	0.047
	Winter	175	2	1.14	01.13	20.32	-	-
Area (Tehsils)	Jahanian	178	9	5.06	15.73	47.97	1.27	0.709
	Kabirwala	172	7	4.07	10.82	40.79	1.02	0.893
	Mian Channu	174	7	4.02	10.82	40.79	1.01	0.893
	Khanewal	176	7	3.98	10.82	40.79	-	-
Animal Keeping	Tethered	366	22	6.01	55.57	86.78	2.51	0.020
	Open	334	8	2.40	13.22	44.43	-	-
Housing System	Closed	233	14	6.01	29.54	64.41	4.45	0.009
	Semi-closed	245	13	5.31	26.63	61.27	3.93	0.031
	Open	222	3	1.35	02.61	24.85	-	-
Hygienic Measures	Very Poor	238	16	6.72	35.59	70.46	6.82	0.002
	Poor	259	12	4.63	23.78	58.07	4.70	0.020
	Good	203	2	0.99	01.13	20.32	-	-
Floor Pattern	Un-Cemented	242	14	5.79	29.54	64.41	4.28	0.018
	Partially-Cemented	236	13	5.51	26.63	61.27	4.08	0.015
	Cemented	222	3	1.35	02.61	24.85	-	-
Watering System	Canal	361	17	4.71	38.73	73.73	1.23	0.646
	Tape	339	13	3.83	26.63	61.27	-	-

not reveal any statistical association of infection with the species of host However in breeds, prevalence of *Anaplasma spp.* was found higher (P<0.05) in cross bred cattle (*B. taurus* x *B. indicus)* than Sahiwal (*B. indicus*) cattle and Kundi than Nili-Ravi buffaloes. Calves and females were more (P<0.05) at risk as compared to adult and males, respectively. *A. marginale* was found statistically higher in cattle and buffaloes than *A. centrale.* Anaplasmosis was chronicled optimum (P<0.05) in summer followed in order by autumn, spring and winter seasons (Figure 2). Infection menace was recorded higher in tethered (P<0.05) than open animals. Animals kept under closed housing system (P<0.05) were found more proned to anaplasmosis than those in semi-closed and open farms in decreasing order. Very Poorly hygiened animals (P<0.05) were in more danger of infection than that of animals kept on farms with poor and good hygienic measures. Animals kept on un-cemented floor were most (P<0.05) susceptible to anaplasmosis followed by those on partially-cemented and cemented floors, respectively. Area and watering system was not found statistically (P>0.05) allied with the disease existence. Table 1 and 2 summarize the association of anaplasmosis with risk factors in cattle and buffaloes of district Khanewal, Punjab, Pakistan, respectively.

DISCUSSION

Anaplasma is the tick-borne rikettsial pathogen of cattle which is found endemic in all six populated continents of the world [21]. Some species of Anaplasma are having zoonotic significance e.g. *A. phagocytophilum* has been reported as 7.5% in Egyptian farmers which were in close proximity of the domestic livestock [22]. Biological transmission is mainly carried by hard ticks particularly by males feeding frequently and not necessarily on the same animal (Intrastadial route) [23]. Transmission of Anaplasma by ticks is highly variable ranging from non-transmission to proficient transmission depending upon the species of tick and parasitic strain [24]. Almost 20 species of ticks are reported as a vector for *Anaplasma* spp. globally including *Hyalomma* spp., *Rhipicephalus* spp., *Boophilus* spp., *Ixodes* spp. and *Demacentor* spp. [7, 25-27]. In ruminants, *Anaplasma* spp. invades erythrocytes and later on reticuloendothelial cells remove them from the circulation causing haemolysis and anaemia [1]. Parasite may also cause rickettsiaemia, accompanied by abortion, fever, decreased milk production, weight loss and sometimes death during acute infections [1, 28]. After the first infection cattle become persistent reservoir and a source of infection for ticks [23, 29]. In agreement with the current study variation in the susceptibility of cattle and buffaloes breeds for anaplasmosis has been reported from other parts of the country [13, 30]. Probable reason for the current results is the immunization of indigenous cattle against parasitic infections due to continuous exposure [31-33].

Anaplasma spp. can cause infections in bovine population of all age categories where severity and mortality rate increases with augmentation of animal age [34]. In divergence with the current study, Khan *et al.* [13] and Atif *et al.* [30] found adult population more prone to bovine anaplasmosis. Probable reasons for the higher disease prevalence in calves of present study are: (a) under developed immune system at young age due to less or absence of exposure to parasitic infections, (b) softer skin resulting in an ease of mouth parts penetration of disease vector and (c) lower disease resistance in young ones as compared to adults [35-39]. Sex of host may influence the extent and severity of the infection [16]. Regarding sex-wise distribution, our findings of statistically higher susceptibility of female animals are not indifferent from those reported elsewhere [30, 40]. Higher prevalence in female population is due to hormonal disturbances due to its use in milk production, draught power and breeding system which pose it to weakened immune system [38, 39].

Endemicity of the disease has been found throughout the year: however, infection becomes epidemic during the hot and humid months of summer providing the most appropriate environmental conditions for the growth and development of tick vectors [41]. Similar association has earlier been reported in Pakistan [42]. Probable reasons for the higher prevalence of anaplasmosis in tethered animals of our study area include: (a) stress exerted due to the rope-tied around neck or hoof, which will lead to generalized immunosuppression and elevated parasitic infections and (b) animal movement is restricted due to confinement that will predispose animals to higher vector infestation and ultimately higher disease prevalence [43].

Hygiene is the most significant determinant that can have great impact on disease surveillance especially of parasitic origin as many a parasitic diseases heavily depend upon the microenvironment for the fulfillment of their life cycle [16, 44]. Probable reasons behind highest disease prevalence in poorly hygienic farms, un-cemented floor pattern and closed housing system include: (a) in the absence of sunlight with unhygienic measures, heaps of dung cakes and stacks of bricks in the closed houses will

provide breeding places for the vector ticks [43] and (b) higher humidity in closed housing system which provides favorable environment for the ovipositioning of female ticks in the cracks and crevices of the walls, roof and floor of house [45].

Recommendations: In the light of current study following recommendations are proposed for the farming and researcher communities: (a) acaricidal therapy before the onset of vector breeding season will prevent tick infestation and hence parasitic transmission, b) special attention towards calves and females with reference to anaplasmosis in specific and tick borne infections in general and c) discouraging the husbandry practices including closed housing, non-cemented floor, tethering and poor hygienic measures.

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