Full Length Research Paper

Prevalence of *Anaplasma marginale*, *Babesia bigemina* and *Theileria annulata* infections among cattle in Sargodha District, Pakistan

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The objective of this study was to investigate the prevalence and distribution of tick-borne diseases in cattle in Sargodha district, Pakistan. Samples were randomly collected each month from selected small holders (n = 30) having 1 to 10 cattle and private livestock farms (n = 4) having ≥ 50 cattle. Indigenous and crossbred cattle of both sexes were sampled. Microscopic examination of the Giemsa stained blood smears revealed an overall prevalence of haemoparasites as 26.86%. *Anaplasma marginale* was the most prevalent (9.71%) hemoparasite of cattle followed by *Theileria annulata* (6.86%) and *Babesia bigemina* (6.57%), respectively. Crossbred cattle (29.1%) were more susceptible to tick-borne diseases (TBDs) as compared to the indigenous cattle (17.7%). Sex wise prevalence indicated that female animals (26%) were more prone to TBDs than males (17%). The highest prevalence of tick-borne diseases was recorded in July except for *T. annulata* which had a peak prevalence in June. The prevalence of tick-transmitted diseases was higher in small holders (31.3%) than large livestock farms (17.5%). Chi square analysis indicated a significant association among different breeds, seasons and farm size to selected tick-borne diseases. Whereas, the difference between different age groups and gender were not statistically significant. It was concluded that TBDs are prevalent in the Sargodha district, Pakistan. There is a need for further epidemiological investigations using advanced serological and molecular techniques.

Key words: *Anaplasma*, *Babesia*, *Theileria*, Sargodha, cattle.

INTRODUCTION

Tick-borne diseases (TBDs) are widely distributed throughout the world especially in tropical and subtropical regions including Pakistan (Jongejan and Uilenberg, 2004; Khan et al., 2004). It has been estimated that TBDs cause US $ 13.9 to 18.7 billion loss per annum and world's 80% cattle population are at risk of ticks and TBDs (de Castro, 1997).

Anaplasmosis is the most prevalent haemo-rickettsial disease of cattle in Pakistan (Khan et al., 2004; Afridi et

al., 2005). The prevalence of *Anaplasma marginale* has been reported to range from 4 to 75.5%, *Anaplasma centrale* 3.5 to 23%, *Babesia bigemina* 1.75 to 7.2%, *Theileria annulata* 0.7 to 24% (Khan et al., 2004; Afridi et al., 2005; Rajput et al., 2005; Ahmad and Hashmi, 2007; Niazi et al., 2008; Al-Khalifa et al., 2009; Aubry and Geale, 2011). 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).

There is little information concerning differences of prevalence of TBDs based on age of animals, gender, breed, season and herd size, which may be of significance

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Table 1. Prevalence of *Anaplasma marginale*, *Babesia bigemina* and *Theileria annulata* using Giemsa stained blood smear among cattle in Sargodha district, Pakistan from August, 2008 to July, 2009.

Parasite	No. examined	No. infected	Prevalence (%)
Anaplasma marginale	350	34	9.71
Babesia bigemina	350	23	6.57
Theileria annulata	350	24	6.86
A. marginale with *B. bigemina*	350	4	1.14
A. marginale with *T. annulata*	350	7	2.00
B. bigemina with *T. annulata*	350	2	0.57
Total	350	94	26.86

in understanding the epidemiology of TBDs and identifying the risk factors for contraction of these diseases. Therefore, this study was designed to investigate the prevalence of major tick-borne diseases in Sargodha district, Pakistan.

MATERIALS AND METHODS

Study area

Sargodha is situated between 32° 10' north latitude and 72° 40' east longitude with average temperature during summer ranging from 25 to 49°C and winter 5 to 23°C with annual rainfall of 526 mm. Agro-ecological mapping based on agricultural development, soil type, vegetation, rain fall and temperature, the Sargodha district is included in Central Mix Cropping Zone of the Punjab, Pakistan (PARC, 2009).

The study area has four seasons that is, autumn (September to October), winter (November to February), spring (March to April) and summer (May to August). Most of the livestock farmers have stall feeding system and poor management practices with usage of unhygienic needles for injecting drugs for milk let down and treatment of generalized diseases.

Sampling strategy

A survey on tick-borne diseases of cattle was conducted at Sargodha district from August, 2008 to July, 2009. A total of 350 blood samples were collected from selected small holders (n = 30) having 1 to 10 cattle and private livestock farms (n = 4) having ≥5 0 cattle using multistage cluster random sampling technique. Animals were sampled in different age groups that is, 1 to 2 year, 2 to 3 year and > 4 years.

All the union councils in the district were included in the sampling frame. A total of 15 union councils, 34 cattle farms (30 small holders and 4 livestock farms) and 350 animals were selected as primary, secondary and elementary sampling units. Sampling unit was indigenous and crossbred cattle of both the sexes. Blood samples (n = 29) were taken in each month except for July and November in which 30 samples were collected per month. Prevalence was estimated using formula (Thrusfield, 1995).

$$P = \frac{d}{n} \times 100$$

Where: P = Prevalence, d = No. of animals found positive, n = Total no. of animals sampled.

Preparation and staining of blood smear

Five milliliter blood samples were collected aseptically in a sterilized syringe from the juggler vein. The blood from ear vein was used to make blood smears for babesiosis. The juggler blood was transferred into test tubes containing EDTA (Improvacuter®, Improve Laboratory Supply, East-Flanders, Belgium).

Thin blood smears were prepared as described by Afridi et al. (2005). The smears were fixed with absolute methanol and stained with diluted Giemsa stain (1:10 ratio) for 25 to 30 min. The smears were rinsed 3 to 4 times with tap water to remove extra stain and air dried. The stained slides were examined at University College of Agriculture, University of Sargodha under oil immersion at 1000x magnification. The blood parasites were identified as described by various OIE publications (OIE, 2004, 2008a, b).

Twenty microscopic fields were observed in search of the selected blood pathogens. If an intra-erythrocytic pathogen was not identified in 20 fields, the animal was considered negative for that pathogen. The results were statistically analyzed by chi square using Statistical Package for Social Services (SPSS) version 13.0. A p-value < 0.05 was considered statistically significant.

RESULTS

Out of 350 samples, 94 were positive for tick-borne pathogens with an overall prevalence of 26.86%. *A. marginale* was the most prevalent (9.71%; 34/350) hemoparasite followed by *T. annulata* (6.86%; 24/350) and *B. bigemina* (6.57%; 23/350 (Table 1). The percentages of tick-borne pathogens among positive cattle were: *A. marginale* (29.06%), *B. bigemina* (19.66%), *T. annulata* (20.51%) and mix infection (23.08%) (Figure 1).

The highest prevalence of *A. marginale* infection (12.40%; 16/129) was found in 2 to 3 year-old animals, while *B. bigemina* and *T. annulata* infection were at 1 to 2 years of age as shown in Table 2. Chi square value indicated that the difference observed between different age groups and gender for *A. marginale, B. bigemina* and *T. annulata* were not statistically significant (Table 3). In contrast, the prevalence of tick-transmitted diseases was statistically significantly higher in crossbred animals
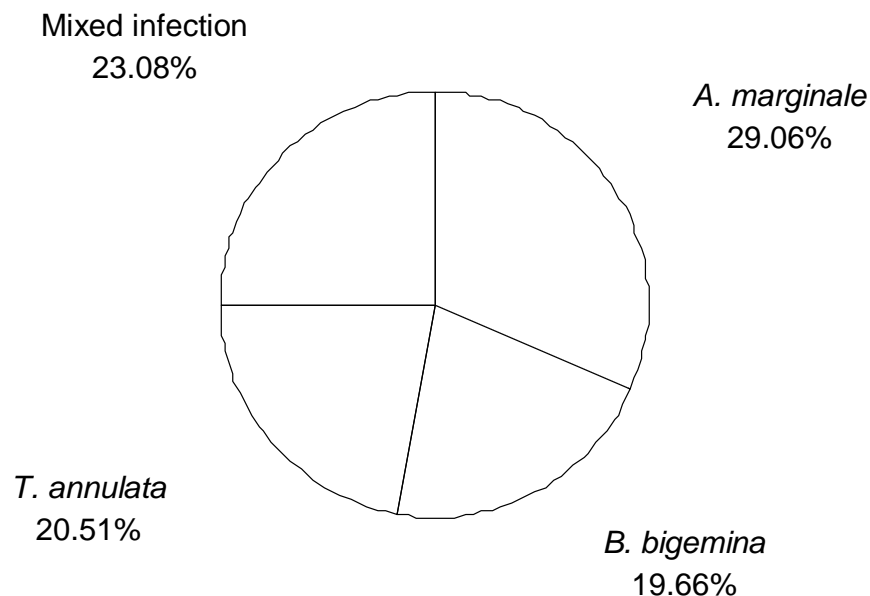
Mixed infection
23.08%

A. marginale
29.06%

T. annulata
20.51%

B. bigemina
19.66%

Figure 1. Distribution of cattle tick borne pathogens in Sargodha district, Pakistan.

Table 2. Prevalence of *Anaplasma marginale*, *Babesia bigemina* and *Theileria annulata* using Giemsa stained blood smears in different age groups of cattle in Sargodha District, Pakistan from August, 2008 to July, 2009.

Age	A. marginale	B. bigemina	T. annulata	Total
1-2	7.23% (6/83)	13.25% (11/83)	10.84% (9/83)	31.33% (26/83)
2-3	12.40% (16/129)	5.43% (7/129)	6.20% (8/129)	24.03% (31/129)
>4	8.70% (12/138)	3.62% (5/138)	5.07% (7/138)	17.39% (24/138)
Total:	9.71% (34/350)	6.57% (23/350)	6.86% (24/350)	23.14% (81/350)

x^2 value = 7.56 (Non-significant); p – value = 0.1088.

Table 3. Prevalence of *Anaplasma marginale*, *Babesia bigemina* and *Theileria annulata* among male and female cattle in Sargodha District, Pakistan from August, 2008 to July, 2009.

Sex	A. marginale	B. bigemina	T. annulata	Total
Male	7.0% (7/100)	6.0% (6/100)	4.0% (4/100)	17% (17/100)
Female	10.8% (27/250)	6.8% (17/250)	8.0% (20/250)	25.6% (64/250)
Total:	9.71% (34/350)	6.57% (23/350)	6.86% (24/350)	23.14 % (81/350)

x^2 value= 0.63 (Non significant); p-value= 0.72.

(28.00%) as compared to indigenous cattle (18.29%) (Table 4).

The highest prevalence of all TBDs of cattle, except *T. annulata* was recorded in the month of July. Whereas *T. annulata* infection was most prevalent in June (Figure 2). The prevalence of tick-transmitted diseases was significantly higher in summer (x^2 = 6.22, p = 0.044) than autumn, winter and spring (Figure 3). Similarly, significantly higher prevalence of tick-transmitted pathogens were recorded in small holders than livestock farms (x^2 = 7.93, df = 2, p = 0.01) (Table 5).

DISCUSSION

The epidemiology of tick-transmitted diseases has not been fully studied in Sargodha district, despite the fact

Table 4. Comparative prevalence of *Anaplasma marginale*, *Babesia bigemina* and *Theileria annulata* using Giemsa stained blood smear among indigenous and crossbred cattle in Sargodha District, Pakistan from August, 2008 to July, 2009.

Breed	*A. marginale*	*B. bigemina*	*T. annulata*	Total
Indigenous	9.14% (16/175)	6.29% (11/175)	2.86% (5/175)	18.29% (32/175)
Cross breed	**10.29% (18/175)	6.86% (12/175)	10.86% (19/175)	28.00% (49/175)
Total:	9.71% (34/350)	6.57% (23/350)	6.86% (24/350)	23.14% (81/350)

x^2 value = 6.49; p – value = 0.039; *(Indigenous x Holstein Friesian).
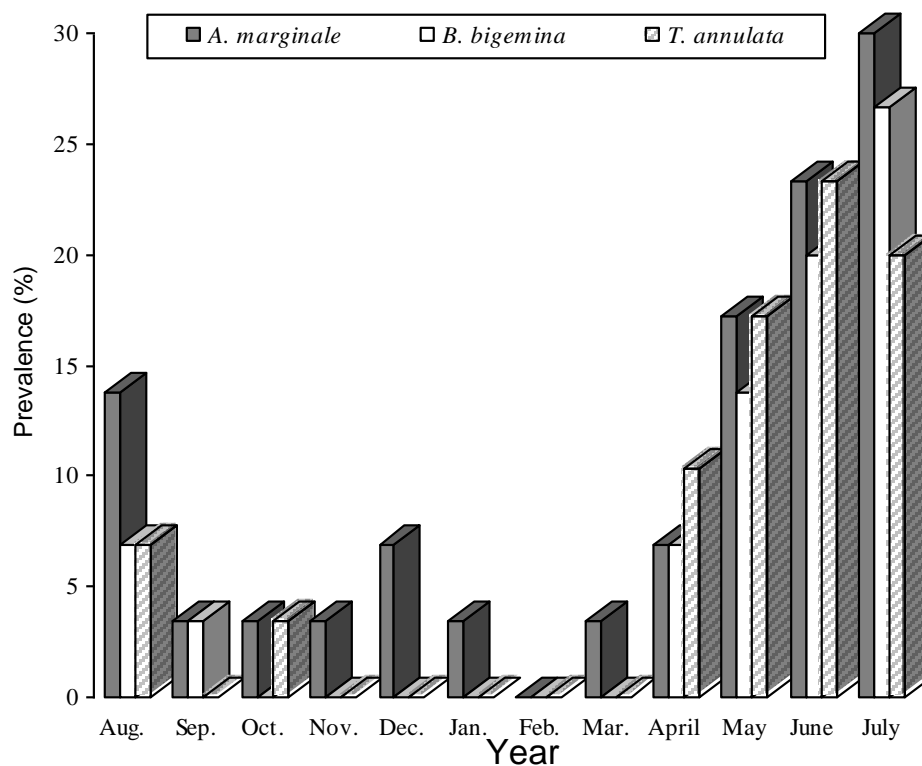

Figure 2. Month wise prevalence of tick-borne pathogens in Sargodha district, Pakistan from August, 2008 to July, 2009.

that they are a major constraint to livestock production and health (Khan et al., 2004). Variable reports on the prevalence of TBDs have been published. The prevalence of *A. marginale* infection in cattle from Peshawar and Hyderabad regions have been recorded as 4.2 and 22%, respectively (Afridi et al., 2005; Rajput et al., 2005). On the contrary, higher prevalence of *A. marginale* (75.7%) has been reported by Khan et al. (2004) at two govern-ment livestock farms located at Attock district and Islamabad, Pakistan as compared to this study.

Zahid et al. (2005) reported the prevalence of *Babesia* sp. to be 5% on a government Livestock Experiment Station, Kasur district. Similarly, Ahmad and Hashmi (2007) have reported the overall occurrence of *B. bigemina* to be 6.6% in cattle from Malakand Agency.

While Afridi et al. (2005) has reported a lower prevalence of 1.75% for *B. bigemina* from Peshawar and adjoining areas as compared to present study. Thirty four animals out of 94 positive cattle showed signs of haemoglobinuria due to intravascular and extravascular haemolysis (Riond et al., 2007) while all the positive animals showed the sings of anaemia.

Khan et al. (2004) and Afridi (2005) have reported comparatively lower (1.42 and 0.70%, respectively) prevalence of *T. annulata* in cattle on microscopic examination of blood smears; whereas, Zahid et al. (2005) mentioned higher (24%) infection of *T. annulata* in exotic Holstein Friesian cattle. The diagnosis is usually based on the identification of tick-borne pathogens on Giemsa stained blood smears but this method is
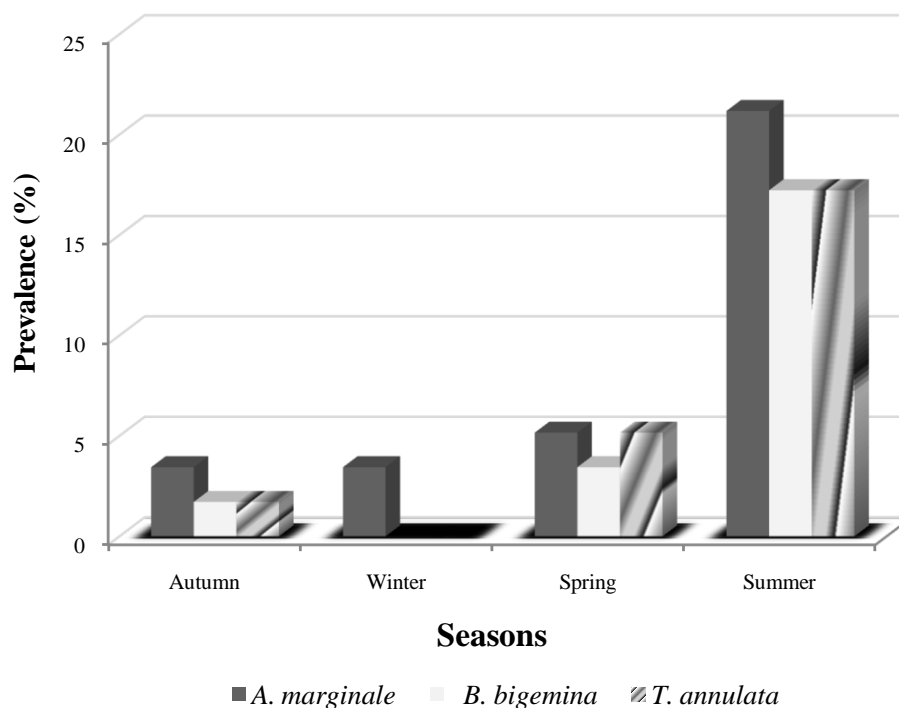
Figure 3. Seasonal prevalence of tick-borne pathogens in Sargodha district, Pakistan from August, 2008 to July, 2009.

Table 5. Comparison of *Anaplasma marginale*, *Babesia bigemina* and *Theileria annulata* prevalence using Giemsa stained blood smear between cattle small holders and livestock farms in Sargodha District, Pakistan from August, 2008 to July, 2009.

Farm size	*A. marginale*	*B. bigemina*	*T. annulata*	Total
Small holder	13.33% (20/150)	12.00% (18/150)	6.67% (10/150)	32.00 (48/150)
Livestock farm	7.00% (14/200)	2.50% (5/200)	7.00% (14/200)	16.50% (33/200)
Total:	9.71% (34/350)	6.57% (23/350)	6.86% (24/350)	23.14% (81/350)

x^2 value = 7.93; p – value = 0.01.

unreliable in the detection of carriers with low levels of parasitaemia or rickettsemia. Thus the prevalence of tick-borne pathogens is likely much higher than that detected in this study.

Previous reports lack distribution of TBDs on the basisof 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 environmental factors (agro-ecological and geo-climatic conditions) influence the prevalence of ticks and tick-borne diseases (Kivaria, 2006).

A higher prevalence of *A. marginale* was apparent in animals of 2 to 3 years of age while *B. bigemina* and *T. annulata* was more prevalent in the 1 to 2 year-old cohort, though these findings were not statistically significant in the present study. Kocan and associates also reviewed that cattle of more than one year of age

are more frequently affected by anaplasmosis (Kocan et al., 2010).

A higher infection rate in females as compared to male cattle has been recorded in the present study. Though this difference was not statistically significant, these findings are in agreement with the results of Durrani (2008) and Rajput et al. (2005) who reported higher prevalence of *T. annulata* and *A. marginale* in female animals. The immunosupression in advanced pregnancy and or lactation in high producing study animals are the possible reasons for the higher prevalence of *A. marginale* in female cattle (Kocan et al., 2010). Moreover, the higher prevalence of TBDs in female animals may be due to the fact that contaminated needles are commonly used for injecting drugs for milk let down.The significant relationship of different breeds on the prevalence of TBDs 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. The European breeds are more susceptible to TBDs due to higher infestation of ticks (Bock et al., 1997, 2004; Glass et al., 2003).

The highest prevalence of *A. marginale* and *B. bigemina* was recorded in July, while *T. annulata* in June. The highest abundance of the ticks was reported in the month of July (Sajid, 2007), whereas *Hyalomma* sp. of ticks were the most abundant in June (Durrani, 2008). *Boophilus microplus* and *Hyalomma anatolicum anatolicum* ticks are the potential vectors of *B. bigemina* and *T. annulata,* respectively in Pakistan (Khan et al., 1994); while the most common vectors of *A. marginale* has not been identified. *B. microplus* ticks are known to be efficient vectors of *A. marginale* in many parts of the world and may serve as a vector in Pakistan.

Ahmad and Hashmi (2007) have reported the highest prevalence of ticks and *B. bigemina* infection in cattle during the month of August in Malakand. This area lies in the cooler, northern high land cooler region of Pakistan. Thus, the tick season is likely delayed as compared to the southern plane regions in which spring generally starts in March. Durrani (2008) also associated the prevalence of *T. annulata* with the tick abundance in June. The prevalence of *A. marginale* in the months of November, December, January and March in the absence of vector ticks suggests mechanical transmission may play a role in the transmission of *A. marginale.*

Milk production is dominated by small holders in Pakistan. It has been estimated that 94.1% farmers have 1 to 10 cattle while only 0.3% farmers have >50 cattle (Agricultural Census Organization, 2006). In the present study, significantly higher prevalence of TBDs was recorded in small holders as compared to large livestock farms. Stall feeding, poor management, lack of tick control practices and inadequate economic sustainability of poor resource small holder farmers may have contributed to the higher prevalence (Swai et al., 2005; Gralen, 2009). Therefore, prevention of small holder farms from tick-borne pathogens would be of crucial importance to reduce poverty and to boost the dairy industry in Pakistan.

Conclusion

It was concluded that bovine TBDs are prevalent in the Sargodha region, Pakistan. There is a need for further investigations using modern serological and molecular techniques for the identification of the carriers of the tick-borne pathogens.

REFERENCES

Ahmad N, Hashmi HA (2007). A comparative study on the incidence of ticks and ticks borne diseases on local and crossbred cattle in Malakand Agency. J. Anim. Pl. Sci., 17(3-4): 56-58.

Afridi ZK, Ahmad I, Khattak GZ, Habib ullah Q, Jamil M (2005). Incidence of anaplasmosis, babesiosis and theileriosis in dairy cattle in Peshawar. Sarhad J. Agric., 21(*3*): 311-316.

Agricultural Census Organization (2006). Pakistan Livestock Census 2006. Statistics Division, Government of Pakistan.

Al-Khalifa MS, Hussein HS, Diab FM, Khalil GM (2009). Blood parasites of livestock in certain regions of Saudi Arabia. Saudi J. Biol. Sci., 16: 63-67.

Aubry, P. Geale DW. (2011).   A review of bovine anaplasmosis. Transbound. Emerg. Dis.*,* 58 (1): 1-30.

Bock RE, de Vos AJ, Kingston TG, McLellan DJ (1997). Effect of breed of cattle on innate resistance to infection with *Babesia bovis, B. bigemina* and *Anaplasma marginale.* Aust. Vet. J., 75: 337-40.

Bock R, Jackson L, de Vos A, Jorgensen W (2004). Babesiosis of cattle. Parasitol., 129: s247-69.Chowdhury S, Hossain MA, Barua SR, Islam S (2006). Occurrence of common blood parasites of cattle in Sirajgonj sadar area of Bangladesh. Bangl. J. Vet. Med., 4(2): 143-145.

de Castro JJ (1997). Sustainable tick and tick borne disease control in livestock improvement in developing countries*.* Vet. Parasitol., 71: 77-97.

Durrani AZ (2008). Epidemiology, serodiagnosis and chemoprophylaxis of theileriosis in cattle. *Ph.D Thesis.* University of Veterinary and Animal Sciences, Lahore, p. 96, 102: 105-122.

Gralen B (2009). Tick-borne diseases in Tajikistan-anaplasmosis, babesiosis and theileriosis. http://ex-psilon.slu.se:8080/archive/00003432/01/Gral%C3%A9n_B_091103.pdf.

Jongejan F, Uilenberg G (2004). The global importance of ticks. Parasitol.*,* 129: S3–S14.

Khan MN, Hayat CS, Hayat B, Iqbal Z (1994). Studies on the vector role of ticks in transmission of protozoan diseases in cattle and buffaloes. Pak. J. Agri. Sci., 31(3): 220-222.

Khan MQ, Zahoor A, Jahangir M, Mirza MA (2004). Prevalence of blood parasites in cattle and buffaloes. Pak. Vet. J., 24(4): 193-195.

Kivaria FM (2006). Estimated direct economic costs associated with tick-borne diseases on cattle in Tanzania. Trop. Anim. Health Prod., 38: 291-299.

Kocan KM, de la Fuente J, Bouin EF, Coetzee JF, Ewing SA (2010). The natural history of *Anaplasma marginale.* Vet. Parasitol., 167: 95-107.

Magona JW, Walubengo J, Olaho-Mukani W, Jonsson NN, Welburn SW and Eisler MC (2011). Spatial variation of tick abundance and seroconversion rates of indigenous cattle to *Anaplasma marginale, Babesia bigemina* and *Therileria parva* infections in Uganda. Exp. Appl. Acarol., 55: 203-213.

Niazi, N., Khan MS, Avais A, Khan JA, Pervez K, Ijaz M (2008). A study on babesiosis in calves at livestock experimental station Qadirabad and adjacent areas of Sahiwal (Pakistan). Pak. J. Agri. Sci., 45(2): 209-211.

OIE (2004). Manual of diagnostic tests and vaccines for terrestrial animals. Bovine babesiosis. Paris, France.

OIE (2008a). Terrestrial Manual, Bovine anaplasmosis. Paris, France.

OIE (2008b). Terrestrial Manual, Theileriosis. Paris, France.

Rajput ZI, Song-hua HU, Arijo AG, Habib M, Khalid M (2005). Comparative study of *Anaplasma* parasites in tick carrying buffaloes and cattle. J. Zhejiang Univ. Sci. B, 6(11): 1057-1062.

PARC (2009). Pakistan Agricultural Research Council, Agro-ecological zones of Punjab.

Sajid MS (2007). Epidemiology, acaricidal resistance of tick population infesting domestic ruminants. Ph.D Thesis. University of Agriculture, Faisalabad, Pakistan, p. 47.

Swai ES, Karimuribo ED, Ogden NH, French NP, Fitzpatrick JL, Bryanto MJ (2005). Seroprevalence estimation and risk factors for *Anaplasma marginale* on small holder dairy farmers in Tanzania. Trop. Anim. Health Prod., 37: 599-610.

Thrusfield M (1995). Veterinary epidemiology, 2nd edition, Blackwell Science, Ltd, Oxford, UK, p. 39-41.

Zahid IA, Latif M, Baloch KB (2005). Incidence and treatment of theileriasis and babesiasis. Pakistan Vet. J., 25(3): 137-139.