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Prevalence and haemato-biochemical changes of tick-borne haemoparasitic diseases in crossbred cattle of Haryana, India

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ABSTRACT

Theileriosis, babesiosis and anaplasmosis are the important tick-borne haemoparasitic diseases (TBHDs) of ruminants, mostly prevalent in the tropics and subtropics, pose a substantial economic losses to dairy industry particularly in developing countries. Here we have discussed the prevalence and haemato-biochemical changes of these TBHDs in eastern Haryana, India. The prevalence of TBHDs in crossbred cattle was studied by screening 10,776 clinically suspected blood samples during the period of July 2010 to June 2015. Among the screened samples, 4,454 animals (41.33%) were found to be TBHDs-positive. Out of 4,454 positive cases, *Theileria annulata, Babesia bigemina, Anaplasma marginale* and mixed infections were reported to be 80.62% (n=3591), 11.52% (n=513), 4.06% (n=180), and 3.82% (n=170), respectively. *T. annulata* was more prevalent during rainy season (37.26%) followed by summer (32.49%) and winter (26.37%); whereas *B. bigemina* and *A. marginale* were more prevalent in summer (6.26% and 1.94%, respectively) followed by winter (4.79% and 1.86%, respectively) and rainy season (3.14% and 1.28%, respectively). Significant decrease of values like Hb, TEC, PCV in TBHDs observed in infected animals compared to healthy control groups, indicated haemolytic anaemia. Hypoglycemia was also observed in the infected group. High activity of ALT and AST was noted among the animals of infected group and may be associated with hepatic injury or due to muscle trauma caused by prolong clinical recumbency. Year-wise an increasing trend of prevalence of tick-borne haemoparasitic diseases and the presence of seasonal variation in occurrence of *T. annulata* and *B. bigemina* infection was observed in the present study. *T. annulata* was found to be the most prevalent and endemic haemoparasites in this region. Findings of the present study have profound clinical significance towards controlling tick-borne diseases as well as improving animal health and productivity.

Key words: *Anaplasma marginale*, *Babesia bigemina,* Eastern Haryana, Haemoparasites, Prevalence, *Theileria annulata*

India contributes a major part of World's livestock genetic resources by sharing approximately 190.9 million cattle (including 39.7 million exotic/crossbred) and 108.7 million buffaloes (Livestock Census 2012). Multi-species tick infestations are common in cattle (especially in crossbred) and buffaloes. Besides transmitting diseases such as theileriosis, babesiosis and anaplasmosis, they also cause extensive damage to the livestock health and production. The global loss due to ticks and tick-borne haemoparasitic diseases (TBHDs) has been estimated to be between US$ 13.9 and 18.7 billion annually whereas India bears a control cost of US$ 498.7 million/annum (Minjauw and McLeod 2003).

The most important tick-borne haemoparasites in cattle are *Babesia*, *Theileria* and *Anaplasma* (Zahid *et al.* 2005). Tropical theileriosis is caused by *Theileria annulata* and transmitted by *Hyalomma* spp. affecting crossbred cattle. The disease is characterized by lymphadenopathy, splenomegaly, fever, anaemia, weakness and loss of body weight (El-Deeb and Younis 2009, Tuli *et al.* 2015 and Maharana *et al*. 2016). Countries like Iran, Turkey, India, and China are at a high risk of this disease, which is incurring heavy economic losses to the livestock owners through mortality and loss in productivity (Razmi 2003).

Babesiosis causes significant morbidity and mortality in cattle worldwide. Protozoan parasites *B. bigemina* and *B. bovis,* causative agents of cattle babesiosis, are transmitted by Ixodid ticks *Rhipicephalus (Boophilus) microplus* which is wide spread in many tropics and subtropics (OIE 2005). Babesiosis results in considerable

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adverse economic impact on cattle industry particularly in developing countries including India (McLeod and Kristjanson 1999, Sharma *et al.* 2013). Infections are characterized by high fever, anorexia and dark brown urine (Yeruham *et al.* 2003).

Another TBHD, anaplasmosis is caused by an obligate intra erythrocytic ricketsial microorganism, *A. marginale*. Sir Arnold Theiler named this disease as gall sickness or yellow bag in 1910 (Theiler 1910, Kocan *et al.* 2010). *Rhipicephalus (Boophilus) microplus* is found to be major transmitting agent (Aubry and Geale 2011) of *A. marginale*. This rickettsial organism is more related to protozoa due to the lack of a traditional cell wall and not being capable of synthesizing lipopolysaccharide and peptidoglycan (Brayton *et al.* 2005). Characteristic features of the disease includes progressive haemolytic anaemia associated with fever, jaundice, decreased milk production, abortions, hyperexcitability and in some cases sudden death (Richey and Palmer 1990, Ashuma *et al.* 2013). The present study was designed to assess the prevalence of haemoprotozoan infections and associated haemato-biochemical changes in pyretic crossbred cows of Eastern Haryana.

MATERIALS AND METHODS

Study area: Haryana, a landlocked state of northern India is located between 27°39' to 30°35' N latitude and between 74°28' and 77°36' E longitude. The climate of Haryana is cold in winter (down to a low of 1° C) and very hot in summer (up to a high of 50° C) and the hottest months are May and June and the coldest being December and January. The state receives an average rainfall of about 650 mm and most of the rainfall occurs in the monsoon season.

Sample collection: Crossbred cows (calves and adults) brought to outpatient department (OPD) of LLRUVAS Referral Veterinary Diagnostic and Extension Centre, Uchani, Karnal during the period of July 2010 to June 2015 and showing clinical signs (fever, anaemia, loss of appetite, cessation of rumination, laboured breathing and haemoglobinurea etc.) similar to TBHDs were included in the present study. All the blood samples were collected in vials with/without anticoagulant (ethylene diamine tetra acetic acid @ 2 mg/ml of blood). Blood smears were prepared immediately after collection from the anticoagulated blood, stained with Giemsa/Leishman stain and examined microscopically for the presence of *T. annulata, B. bigemina* and *A. marginale*. The haemoprotozoa were identified to species level as per morphological characters (Soulsby 1982). Haematological parameters including haemoglobin (Hb g/dl), packed cell volume (PCV%) and total erythrocyte cells (TEC × 10^6/µl) were estimated as per Schalm (1965). Coagulated random blood samples of infected (n=100) and healthy crossbred cattle (n=10) were centrifuged at 5,000 rpm for 15 min and the supernatant was collected for biochemical estimations. The biochemical parameters, viz. blood glucose, alanine transferase (ALT) and aspartate transferase (AST) were analysed by fully automatic biochemistry analyzer (EM Destiny 180, Erba). Annual rainfall data were obtained from local observatory of CCS, Haryana Agricultural University, Regional Research Station, Uchani (Karnal). Prevalence was estimated using formula as described by Thursfield (1995)

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

where, p, prevalence; d, number of animals found positive; n, total number of animals sampled.

The data of haemoprotozoan infections were pooled season-wise i.e., winter (November to February), summer (March to June) and rainy (July to October). The difference in mean of biochemical parameters between groups and prevalence of infections between seasons were compared using one-way ANOVA of SPSS software version 24.

RESULTS AND DISCUSSION

Out of 10,776 crossbred blood samples analysed, 4,454 samples were found positive for tick-borne pathogens with an overall prevalence of 41.33%. *T. annulata* (33.32%) was the most prevalent haemoparasites followed by *B. bigemina* (4.76%), *A. marginale* (1.67%) and mixed infection (1.58%) (Table 1). The tick-borne pathogens among positive cattle samples were found to be 80.62, 11.52, 4.04 and 3.82% for *T. annulata, B. bigemina, A. marginale* and mixed infections, respectively. Mixed infection was recorded in 170 samples; where maximum co-infection was associated with *T. annulata* + *B. bigemina* (68.24%, n=116) followed by *T. annulata* + *A. marginale* (25.88%, n=44) and *A. marginale* + *B. bigemina* (4.7%, n=8). Two samples (1.18%) were observed to have infected with all the three haemoparasites i.e., *T. annulata* + *B. bigemina* + *A. marginale.* Further analysis revealed a continuous increment of the prevalence of *T. annulata* in crossbred cows from July 2010 to June 2015 (21.86 to 44.8%) except in 2011–12 (14.46%). On the other hand, a regular increase was observed for *B. bigemina*, *A. marginale* and mixed infection from July 2010 to June 2015 (1.08 to 6.9%; 1.08 to 2.2% and 0.29 to 2.4% respectively) (Fig. 1). Year-wise prevalence data showed an increasing trend of haemoparasitic infections at Eastern Haryana. Earlier studies from cross-bred cows of Northern India including Haryana (Yadav *et al.* 1985, Chaudhri *et al.* 2013), Punjab (Aulakh *et al.* 2005) and Himachal Pradesh (Jithendaran 1997), bovine calves of semi-arid region from Rajasthan (Godara *et al.* 2010), cattle from Northern Kerala (Nair *et al.* 2011) and crossbred cattle and buffaloes of Kaira and Anand districts of Gujarat (Vohra *et al.* 2012) showed that TBHDs are widely prevalent in large population of dairy animals and thus adversely affect milk production to a larger extent. High prevalence of haemoparasitic diseases may be linked to increased activity of corresponding tick vectors during summer and rainy seasons (Sangwan *et al.* 1995, Ananda *et al.* 2009, Vohra *et al.* 2012 and Kumar *et al.* 2015). A considerable seasonal variation was observed with the occurrence of TBHDs in animals.

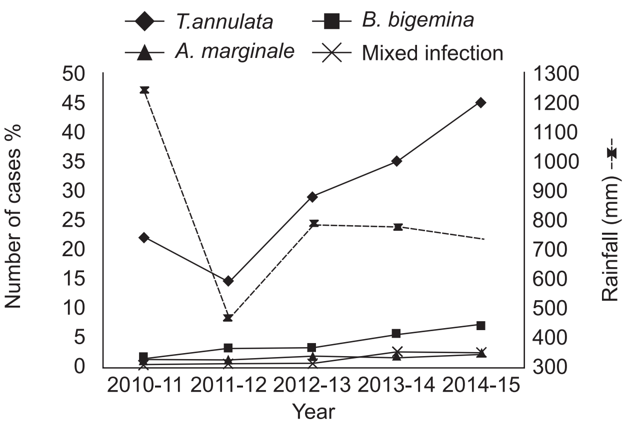

Fig. 1. Prevalence of tick-borne haemoparasitic diseases in crossbred cattle recorded from July 2010 to June 2015 in eastern region of Haryana.

Significantly higher (P<0.05) prevalence of *T. annulata* was recorded in rainy (37.26%) and summer (32.49%) compared to winter (26.61%); whereas prevalence of *B. bigemina* and *A. marginale* were found highest in summer (6.26% and 1.94%, respectively) (Table 1). The present results are in accordance with earlier studies (Vohra *et al*. 2012 and Roy *et al*. 2004); where they reported highest prevalence in monsoon months. On the contrary, Chakraborty (1993) and Velusamy *et al*. (2014) reported a higher prevalence of theileriosis during summer (17.64% and 14.4%) followed by rainy (7.32% and 13.8%) and winter (5% and 11.5%), respectively. Theileriosis was reported from various parts of India; 16.64% in Western Tamil Nadu (Velusamy *et al.* 2014), 16% in Northern Kerala (Nair *et al*. 2011), 17.7% in Karnataka (Muraleedharan *et al*. 1994), and 45.4% in Dehradun (Kohli *et al*. 2014). The results of present study further showed a considerable higher prevalence of *T. annulata* in Northern India compared to Southern and Eastern parts of India.

Prevelance of *B. bigemina* was significantly (P<0.05) higher during summer (6.26%) compared to rainy (3.14%) and winter (4.79%) seasons. In other reports, higher prevalence was observed in rainy season followed by summer and winter (Bhikane *et al*. 2001, Shekar and Haque 2007, Velusamy 2014). However, in the present study, no significant (P>0.05) seasonal influence was observed on anaplasmosis and mixed infection (Table 1). These differences observed in the prevalence may be due to the high abundance of tick vector, different geographical locations, presence of ideal breeding microenvironment (high temperature and humidity) suitable for survival and breeding of ticks (Magona *et al*. 2011).

Haryana comes under low rainfall region and receives an annual rainfall in between 50 and 100 cm. The year 2011–12 was rainfall deficient and received an annual rainfall (460.8 mm) below the lower limit. When rainfall data are used along with year-wise prevalence, a sharp decline was observed in the *T. annulata* infection during the rainfall deficient year 2011–12 (Fig. 1). To see the effect of rainfall over TBHDs, season-wise data were analysed over the years

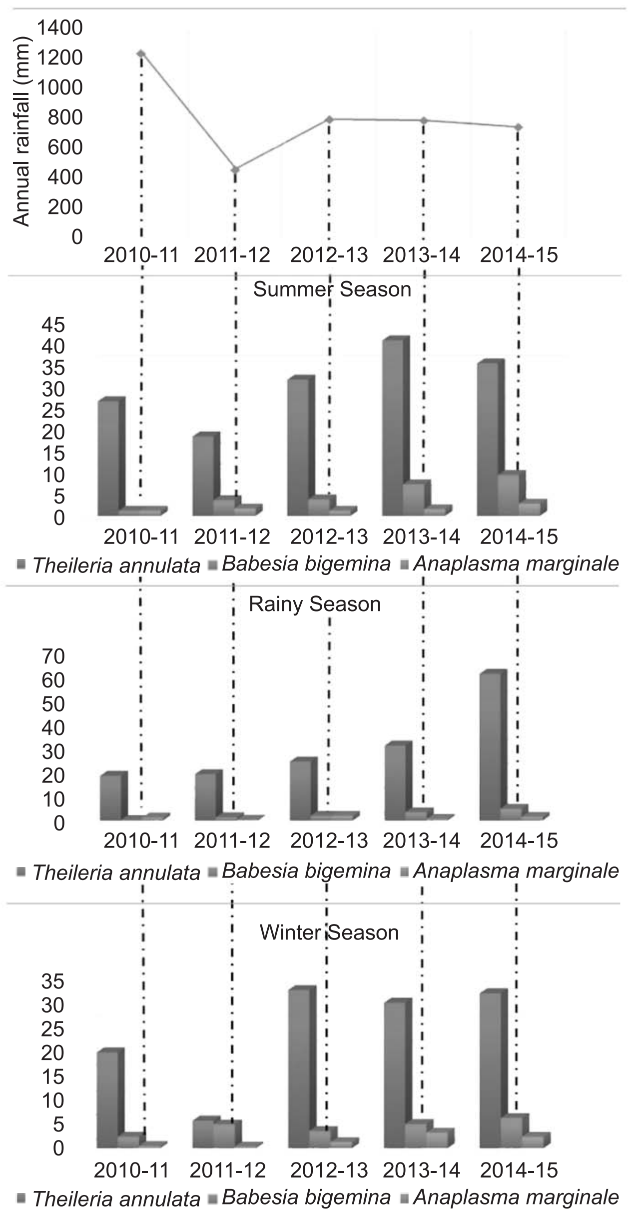

Fig. 2. Effect of annual rainfall on seasonal pattern of *Theileria annulata, Babesia bigemina* and *Anaplasma marginale* infection among cattle of Eastern Haryana from July 2010–June 2015.

(Fig. 2). Although a decline was noticed during rainy season (2011–12), however a sharp decline was observed in the *T. annulata* infection during winter of 2011–12. However, no such decline could be observed in *B. bigemina* and *A. marginale* infections. It was reported that *Hyalomma anatolicum,* transmitting agent of *T. annulata,* is a very common hard tick species abundant in the North India, including Haryana, showing maximum activity during the summer and post rainy season (Chhillar *et al.* 2014). The decrease of *T. annulata* infection in rainfall deficient year 2011–12 may be due to reduction of carrier tick population or their activities, post monsoon. Whereas, *Rhipicephalus (Boophilus) microplus,* transmitting tick of *B. bigemina* and *A. marginale,* is found throughout the year infesting cattle

Table 1. Seasonal pattern of *Theileria annulata, Babesia bigemina and Anaplasma marginale* infection among cattle of Eastern Haryana from July 2010-June 2015

Season	Month	Number of blood sample examined	*T.annulata*	*B. bigemina*	*A. marginale*	Mixed	TBDs affected cows
Summer	March	649	198	36	17	7	258
	April	1054	279	57	25	19	380
	May	1263	442	99	19	39	599
	June	1552	549	91	27	32	699
Total		4518	1468	283	88	97	1936
prevalence			32.49^{a}	6.26^{a}	1.94^{ns}	2.14^{ns}	42.85^{a}
Rainy	July	1218	495	33	24	11	563
	August	1278	492	25	17	8	542
	September	1082	359	40	8	18	425
	October	715	254	37	6	3	300
Total		4293	1600	135	55	40	1830
prevalence			37.26^{a}	3.14^{b}	1.28^{ns}	0.93^{ns}	42.63^{a}
Winter	November	538	171	19	4	8	202
	December	545	138	26	13	7	184
	January	415	82	24	15	7	128
	February	467	132	26	5	11	174
Total		1965	523	95	37	33	688
prevalence			26.61^{b}	4.83^{b}	1.88^{ns}	1.67^{ns}	35.01^{b}
Total of 2010-15		10776	3591	513	180	170	4454
Overall prevalence			33.32	4.76	1.67	1.58	41.33

Mean with different superscripts are significantly different (P<0.05); ns, nonsignificant.

Table 2. Haemato-biochemical parameters of tick-borne haemoparasitic disease (TBHD) affected animals

Parameters	Healthy animals Mean±SE (n=10)	TBHD affected animals Mean±SE (n=100)	P-value
Hb (g/dl)	12.02±0.21	5.11±0.18**	<0.00001
PCV (%)	35.6±1.02	15.17±0.58**	<0.00001
TEC (× 10^6/µl)	7.04±0.17	3.55±0.13**	<0.00001
Glucose (mg/dl)	60.8±1.59	53.32±1.07*	0.030
ALT (U/L)	32.6±1.99	100.27±8.21*	0.010
AST (U/L)	76.9±2.27	136.94±8.7*	0.030

*TBHD affected and healthy cattle significantly different at P<0.05. **Significant at P<0.001. Hb, Haemoglobin; PCV, packed cell volume; TEC, total erythrocyte count; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

and buffalo, but preferring exotic and crossbred cattle (Chhillar *et al.* 2014) may be less affected by rainfall.

Significantly (P<0.00001) low levels of Hb, PCV and TEC were observed in TBHD affected animals compared to normal healthy animals (Table 2) indicating haemolytic anaemia. High activities of alanine transferase and aspartate transferase enzymes were recorded in infected group compared to healthy control (P<0.05) and are closely associated with hepatic injury caused by the protozoa (Forsyth *et al.* 1999). The significant (P<0.05) increase in the serum AST and ALT activities may also be due to muscle trauma caused by prolonged clinical recumbency in theileriosis (Sandhu *et al*. 1998). Similar findings were observed in *T. annulata* infected crossbred cattle (Ganguly *et al*. 2015). Significant decrease in blood glucose concentration (P<0.05) observed in the infected animals could be due to the utilization of glucose by parasites and damage to the liver in large ruminants infected with tick-borne pathogens. The haematological values were adversely affected in positive cases. In severely infected cases, Hb level was reduced to 3 g/dl. The TEC and PCV were decreased to 1.99 ×10^6/µl and 10% respectively (data not shown).This might be due to damage caused by the organisms inside the RBC's during their multiplication.

Similarly, Muraleedharan *et al*. (2005) and Ganguly *et al*. (2015) have reported low levels of Hb and TEC in animals infected with *T. annulata*. Similar results were obtained in experimentally induced cases of bovine tropical theileriosis (Mehta *et al*. 1988).

In summary, the current study has shown an increasing trend of prevalence of tick-borne haemoparasitic diseases from 2010–2015 and the presence of seasonal variation in occurrence of *T. annulata* and *B. bigemina* infection in Eastern Haryana. The study also suggested that *T.annulata* is the most prevalent and endemic hamoparasites in this region. Findings of the present investigation have profound clinical significance towards controlling tick-borne diseases as well as improving animal health and productivity.

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