

Original Research Article

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## Prevalence of Haemoparasites of Cattle in Four Districts of Assam, India and Bordering Bhutan

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### ABSTRACT

#### Keywords

Cattle,  
Haemoprotozoan,  
*Theileria orientalis*,  
*Babesia bigemina*,  
*Anaplasma marginale*, Bhutan,  
Blood smear

#### Article Info

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The present investigation was done for one year to record the prevalence of haemoparasite species in crossbred and domestic cattle along the four Indo-Bhutan border districts of Assam. Microscopic examination of blood smears from 533 cattle resulted in overall prevalence of 67.35% and detection of three species, viz. *Theileria orientalis* (62.85%), *Babesia bigemina* (2.62%) and *Anaplasma marginale* (1.87%). Infection was found higher in crossbred (75.32%) than in indigenous cattle (66.00%), which was statistically highly significant ( $P < 0.01$ ). Age wise, prevalence of haemoparasites were found highest in adult cattle  $>3$  years (80.42%) followed by young (68.11%) and lowest in calves (54.85%). Breed wise, higher prevalence was recorded in crossbred (75.32%) than in indigenous cattle (66.00%). Sex wise, females revealed higher prevalence of haemoparasites (74.02%) than the male counterpart (58.22%). Cattle and other animals are being regularly traded across the porous Indo-Bhutan border areas. However, there is no record on the haemoparasitic diseases prevailing in the border districts. The various factors related to prevalence of haemoparasite species of cattle in these areas are discussed.

### Introduction

Livestock is an important subsector of Indian Agriculture economy with cattle (199.10 million) and buffalo (105.30 million) accounting for 16.24% and 56.90% respectively of world bovine population (Livestock census 2007, Govt. of India). Assam situated in India's Northeast region is characterized by low milk producing cattle with average productivity of 1.34 L/ day against the all-India average of 2.77 L/day.

There still exists a huge gap in the production and consumption of milk and other livestock products. Apart from the indigenous cattle, crossbreds are being produced by up-gradation of indigenous animals with Jersey and Holstein Friesian exotic germ plasms.

Arthropod borne haemoprotozoan and haemoretic diseases like trypanosomosis, theileriosis, babesiosis, anaplasmosis and ehrlichiosis cause huge economic loss to the livestock industry

throughout the world, responsible for high degree of morbidity and leading to mortality or long term debilitating effects causing anaemia, emaciation and reduction in milk production in both exotic and crossbred cattle (PD-ADMAS, 2005-06). Further, recovered animals become carriers, and are a potential source of infection to healthy susceptible population (Callow, 1984). In India, the cost of tick and tick borne disease (TTBD) control in animals has been estimated to be US \$ 498.7 million per annum (Minjauw and McLeod, 2003).

Theileriosis caused by *Theileria annulata* is an important disease and in India, the annual loss reported is approximately US\$ 800 million. *Theileria orientalis*, another parasite under the genus and previously considered to be non-pathogenic or mildly pathogenic, occur in all continents, are transmitted mainly by *Amblyomma*, *Rhipicephalus* and *Haemaphysalis* ticks and cause benign theileriosis (Uilenberg, 1981). There was no local knowledge on prevalence of haemoparasite other than *Babesia* and *Anaplasma* in cattle of Assam till the report of Kakati (2013) who recorded predominance of *Boophilus microplus* tick vector and incidence of *Theileria orientalis* besides *B. bigemina* and *A. marginale* in indigenous and crossbred cattle and record of mortality among crossbreds but no *T. annulata*. Bovine anaplasmosis, caused by *A. marginale*, is an important haemoretic disease transmitted biologically by ticks and mechanically by biting flies and blood contaminated fomites causing significant economic losses in tropical and subtropical areas of world (Ristic, 1981).

The Northeast India represents the transition between India, Myanmar, Bangladesh, China and Bhutan and is the geographical gateway for much of flora and fauna (Rai, 2008). Animal diseases often transcend international

boundaries (Trans Border Animal Diseases-TADs) through unabated movement of animals and can become the cause of national emergencies so far the animal and human health is concerned. Bhutan, known as the “Thunder Dragon Country” is a tiny independent kingdom bordered in the east, west and south by the Indian states of Arunachal Pradesh, Sikkim, Assam and West Bengal, while in the north by China. Among diseases of cattle, tick borne diseases as babesiosis, theileriosis and anaplasmosis are the major recognized problem in Bhutan (Phanchung *et al.*, 2012; Tshering and Dorji, 2013). The border trade between the India and Bhutan takes place through several recognized passes or duars. Assam is the major state of which six districts such as Kokrajhr, Bongaigaon, Chirang, Baksa, Udalguri and Sonitpur covering approximately 1000 square miles area share boundary with Bhutan.

Livestock for milk production and draught purpose are being regularly traded through the porous border, which are considered to be the risk factors for transmission of various diseases. Therefore studies on these organisms in these porous borders are of great importance in the monitoring and surveillance of trans-boundary animal diseases.

## Materials and Methods

### Study area

The present study was carried out in four districts of Assam (26.24°-26.6897°N Latitude and 90.16°-91.9099°E Longitude) namely, Kokrajhar, Chirang, Baksa and Udalguri along the Indo-Bhutan border areas for one year w.e.f. April 2016 to March 2017. The environmental temperature ranged from 8° to 15°C during winter and 35° to 38° C during the summer.

## Study design

A total of 533 cattle (456 indigenous and 77 crossbred) were considered for the prevalence of haemoprotozoan and haemoreticulous diseases in the study area. Collection of animal level data such as age, sex, breed, and husbandry practices were collected by interviewing the owners/farmers. According to age, animals were categorized into calves (<1 year), young (1-3 years) and adult (>3 years). Indigenous (*Bos indicus*) and crossbred (Holstein Friesian, Jersey, *Bos taurus* X *Bos indicus*) cattle of either sex were selected randomly on the basis of their availability. The crossbred animals were stall-fed, kept on concrete/ semi concrete floors whereas the indigenous animals were of open grazed type and raised on muddy floor of the sheds.

## Microscopic detection of haemoparasites

Anticoagulated blood was collected from 128 animals in Kokrajhar district, 139 from Chirang, 146 from Baksa and 120 from Udalguri district, thin blood smears prepared and stained with Giemsa stain. Smears were then examined under high power (40X) and oil immersion objective (100X) of a compound microscope for detection of parasite within the red blood cells and lymphocytes. The parasites were identified on the basis of their characteristic morphology (Levine, 1978; Soulsby, 1982). Percent parasitaemia (No. of parasitized cell /Total no. of respective cell x 100 = % parasitaemia) in positive cases was estimated by counting at least 10 randomly chosen microscopic fields containing a single layer of non overlapping cells under oil immersion objective. Failure to detect parasite in a blood smear even after examination of at least 500 oil immersion fields over a time period of 20-30 minutes was recorded as negative blood sample.

## Statistical analysis

SAS Enterprise Guide 4.3 software program was employed for the data analysis using Chi-square ( $\chi^2$ ) test. The results were expressed in percentage with p-value and the significance was determined with p value of <0.05. Odds Ratio was calculated according to the formula given by Schlesselman (1982).

## Results and Discussion

### Prevalence of haemoparasites in crossbred and indigenous cattle

In the present study, the prevalence of haemoparasites in cattle by blood smear examination was found to be 67.35%, either in single or mixed infections (Table 1). Three species of blood parasites were identified as *Theileria orientalis* (62.85%) followed by *Anaplasma marginale* (2.62%) and *Babesia bigemina* (1.87%). No case of *Theileria annulata*, *Babesia bovis* and *Trypanosoma evansi* was recorded. Statistically, the difference in species wise prevalence of haemoparasites was highly significant (P<0.01). Overall prevalence was recorded highest in Chirang district (71.22%) followed by Kokrajhar (70.31%), Baksa (67.12%) and Udalguri (60.00%), the prevalence was statistically highly significant (P<0.01).

Varying prevalence of haemoprotozoan parasites was reported by workers from India and abroad (3.9% by Sebele *et al.*, 2015 in Ethiopia; 22.9% by Singh *et al.*, 2012 in Ludhiana; 27.2% by Kohli *et al.*, 2014 in Dehradun; 43.18% by Ananda *et al.*, 2009 in Bangalore; 56.89% by Kakati, 2013 in Assam; 76.85% by Reetha *et al.*, 2012 in Tamil Nadu). There are very scanty reports on *T. orientalis* in India which might be due to the fact that this parasite responsible for Oriental theileriosis was earlier considered as a mild one and had usually no ill effects in cattle.

**Table.1** Prevalence of haemoparasites in crossbred and indigenous cattle from Indo-Bhutan border districts of Assam

Haemoparasite species recorded	Kokrajhar		Chirang		Baksa		Udalguri		Total		Odds Ratio	Significance level ( $\chi^2$ )
	Cross-bred (n=18)	Indigenous (n=110)	Cross-bred (n=25)	Indigenous (n=114)	Cross-bred (n=19)	Indigenous (127)	Cross-bred (n=15)	Indigenous (n=105)	Cross-bred (n=77)	Indigenous (n=456)		
	No. positive (%)	No. positive (%)	No. positive (%)	No. positive (%)	No. positive (%)	No. positive (%)	No. positive (%)	No. positive (%)	No. positive (%)	No. positive (%)		
<i>Theileria orientalis</i>	12 (66.67)	72 (65.45)	20* (80.00)	70 (61.40)	10 (52.63)	84 (66.14)	10 (66.67)	57 (54.28)	52 (67.53)	283 (62.85)	Cross-bred Vs Indigenous =1.57	P < 0.001
<i>Babesia bigemina</i>	2 (11.11)	2 (1.81)	0 (0.00)	1 (0.87)	0 (0.00)	2 (1.57)	1 (6.67)	2 (1.90)	3 (3.89)	7 (1.53)		
<i>Anaplasma marginale</i>	0 (0.00)	2 (1.81)	0 (0.00)	8 (7.01)	1 (5.26)	1 (0.78)	2 (3.33)	0 (0.00)	3 (3.89)	11 (2.41)		
<b>Total</b>	<b>14 (77.77)</b>	<b>76 (69.09)</b>	<b>20 (80.00)</b>	<b>79 (69.29)</b>	<b>11 (57.89)</b>	<b>87 (68.50)</b>	<b>13 (86.66)</b>	<b>59 (56.19)</b>	<b>58 (75.32)</b>	<b>301 (66.00)</b>		
<b>Overall prevalence</b>	<b>90 (70.31)</b>		<b>99 (71.22)</b>		<b>98 (67.12)</b>		<b>72 (60.00)</b>		<b>359 (67.35)</b>			

Highly significant

**Table.2** Prevalence of haemoparasites in relation to sex of cattle in Indo-Bhutan border districts of Assam

Haemoparasite species recorded	Kokrajhar		Chirang		Baksa		Udalguri		Total		Significance level ( $\chi^2$ )
	Male (n=54)	Female (n=74)	Male ((n=61)	Female (n=78)	Male (n=59)	Female (n=87)	Male (n=51)	Female (n=69)	Male (n=225)	Female (n=308)	
	No. positive (%)	No. positive (%)	No. positive (%)	No. positive (%)	No. positive (%)	No. positive (%)	No. positive (%)	No. positive (%)	No. positive (%)	No. positive (%)	
<i>Theileria orientalis</i>	25 (46.29)	59 (79.72)	41 (67.21)	49 (62.82)	39 (66.10)	55 (63.21)	23 (45.09)	44 (63.76)	128 (56.88)	207 (67.20)	P<0.0001
<i>Babesia bigemina</i>	0 (0.00)	4 (5.40)	0 (0.00)	1 (1.28)	0 (0.00)	2 (2.29)	1 (1.96)	2 (2.89)	1 (0.44)	9 (2.92)	
<i>Anaplasma marginale</i>	0 (0.00)	2 (2.70)	1 (1.63)	7 (8.97)	0 (0.00)	2 (2.29)	1 (1.96)	1 (1.44)	2 (0.88)	12 (3.89)	
<b>Total</b>	<b>25 (46.29)</b>	<b>65 (87.82)</b>	<b>42 (68.85)</b>	<b>57 (73.07)</b>	<b>39 (66.10)</b>	<b>59 (67.81)</b>	<b>25 (49.01)</b>	<b>47 (68.11)</b>	<b>131 (58.22)</b>	<b>228 (74.02)</b>	
<b>Overall prevalence</b>	<b>90 (70.31)</b>		<b>99 (71.22)</b>		<b>98 (67.12)</b>		<b>72 (60.00)</b>		<b>359 (67.35)</b>		

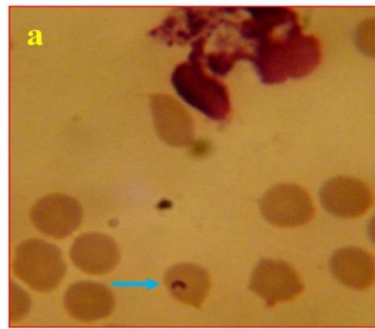
Highly significant

**Table.3** Prevalence of haemoparasitic infection in relation to age of cattle

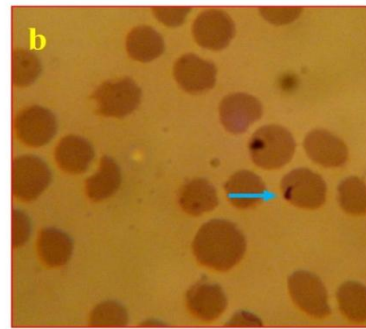
District	Age group n=No. examined	Haemoparasites recorded			Total No. positive (%)	Odds Ratio	Significance level ( $\chi^2$ )
		<i>Theileria orientalis</i> No.positive (%)	<i>Babesia bigemina</i> No.positive (%)	<i>Anaplasma marginale</i> No. positive (%)			
<b>Kokrajhar</b>	Calf (n=50)	17 (34.00)	0 (0.00)	0 (0.00)	17 (34.00)	Calf Vs Young =1.22  Adult Vs Calf =3.58	P<0.001
	Young (n=31)	25 (70.96)	2 (6.45)	0 (0.00)	27 (77.41)		
	Adult (n=47)	42 (89.36)	2 (4.25)	2 (4.25)	46 (97.87)		
<b>Chirang</b>	Calf (n=51)	33 (64.70)	0 (0.00)	0 (0.00)	33 (64.70)		
	Young (n=35)	21 (60.00)	0 (0.00)	3 (8.57)	24 (68.57)		
	Adult (n=53)	36 (67.92)	1 (1.88)	5 (9.43)	42 (79.24)		
<b>Baksa</b>	Calf (n=54)	33 (61.11)	0 (0.00)	0 (0.00)	33 (61.11)		
	Young (n=40)	27 (67.50)	0 (0.00)	0 (0.00)	27 (67.50)		
	Adult (n=52)	34 (65.38)	2 (3.84)	2 (3.84)	38 (73.07)		
<b>Udalguri</b>	Calf (n=51)	30 (58.82)	0 (0.00)	0 (0.00)	30 (58.82)		
	Young (n=32)	15 (46.87)	1 (3.12)	0 (0.00)	16 (50.00)		
	Adult (n=37)	23 (62.16)	1 (2.70)	2 (5.40)	26 (70.27)		
<b>Total</b>	<b>Calf (n=206)</b>	<b>113 (54.85)</b>	<b>0 (0.00)</b>	<b>0 (0.00)</b>	<b>113 (54.85)</b>		
	<b>Young (n=138)</b>	<b>88 (63.76)</b>	<b>3 (2.17)</b>	<b>3 (2.17)</b>	<b>94 (68.11)</b>		
	<b>Adult (n=189)</b>	<b>135 (71.42)</b>	<b>6 (3.17)</b>	<b>11 (5.82)</b>	<b>152 (80.42)</b>		

Highly significant

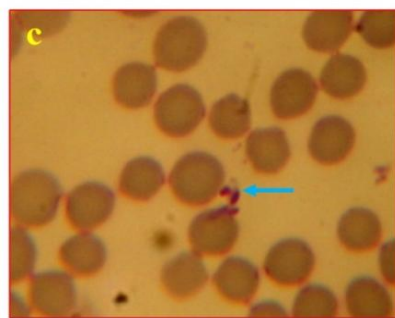
**Fig.1** (a-h): Erythrocytic parasites of *Theileria orientalis* (1000 X magnification)



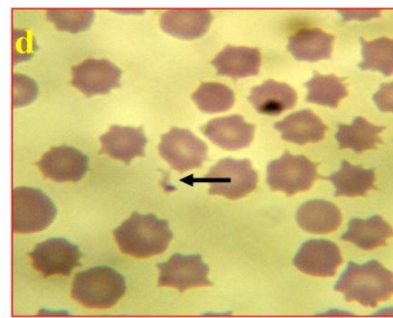
**(a) Crescent form/Ring form**



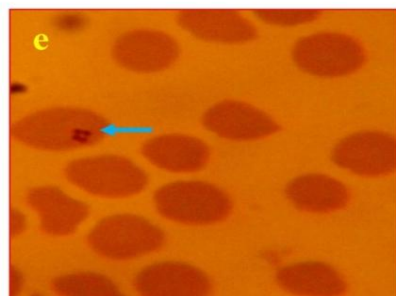
**(b) Tail-like finger form**



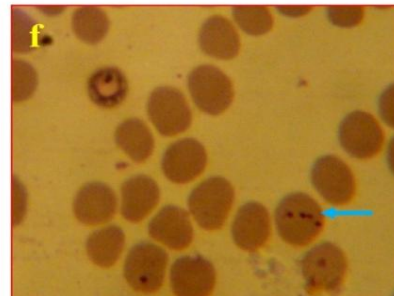
**(c) Extra cellular form**



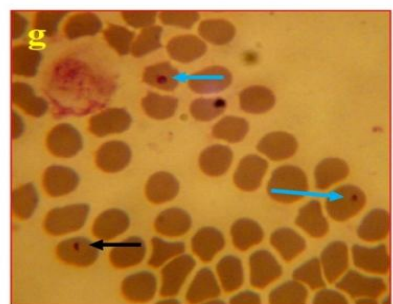
**(d) Extra cellular form**



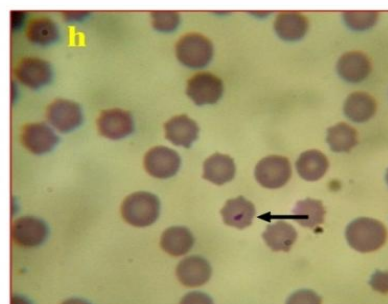
**(e) Tetrad form**



**(f) Dividing form**

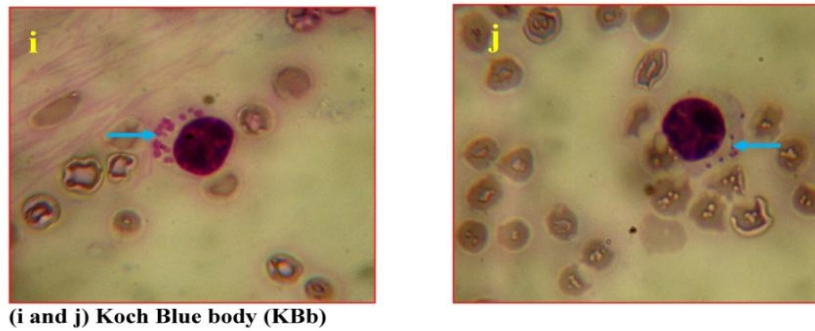


**(g) Dot form (blue arrow), Rod like/  
Bar form (black arrow)**

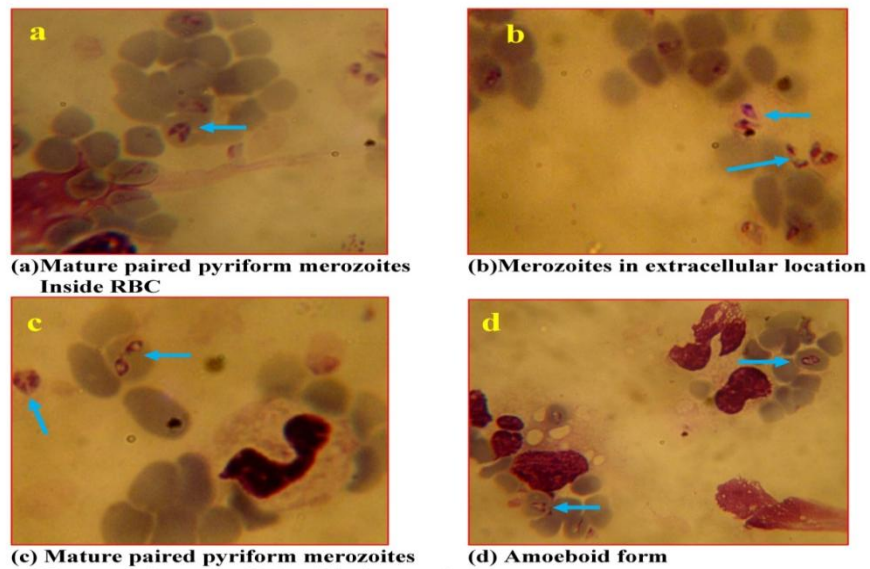


**(h) Comma like form**

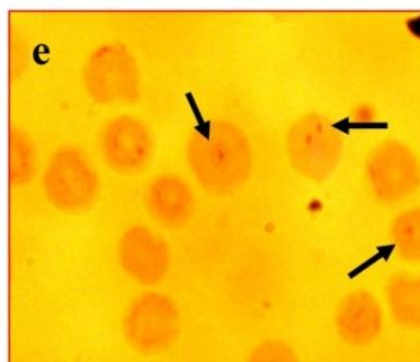
**Fig.1 (i-j)** Giemsa stained blood smear of cattle showing intralymphocytic parasites of *Theileria orientalis*, KBb (1000 X magnification)



**Fig.2 (a-d):** Giemsa stained blood smear of cattle showing *Babesia bigemina* (1000 X)



**Fig.2 (e):** *Anaplasma marginale* inside RBC (1000X)



However, Kakati (2013) observed outbreaks of theileriosis with mortality in cattle of Assam due to *T. orientalis* and confirmed its virulence similar to recent reports made by Aparna *et al.*, (2011) from India and many other countries like Australia (Eamens *et al.*, 2013), Michigan, USA (Bayugar *et al.*, 2002) and Japan (Yokoyama *et al.*, 2010). Several workers from different parts of India recorded in addition to the above three species, the prevalence of *T. annulata* and *T. evansi* in cattle and buffaloes (Das and Sharma, 1991; Ananda *et al.*, 2009; Sahoo *et al.*, 2012).

*T. orientalis* inside the erythrocytes were found in different forms such as rod, bar, comma, ring (annular), dot, crescent shaped, finger like, tail like with trailing cytoplasm and tetrad (dividing form). Merozoites were also found extracellularly (Fig.1 a-h). Schizont of *T. orientalis* (Koch Blue Body) was also detected in the lymphocytes in a few cases (Fig.1 i-j). Piroplasms of *B. bigemina* varied in shape from ovoid or vacuolar forms, amoeboid to typically paired pyriforms with acute angle inside the erythrocytes and also in extracellular location (Fig.2 a-d).

Inclusion bodies of *A. marginale* were dot shaped appearing in the margin or periphery of erythrocytes (Fig.2 e). Aparna *et al.*, (2011), Kakati (2013) and Anupama *et al.* (2015) also reported similar forms of *T. orientalis*. *Babesia* organisms with different morphology were similar to the description given by Soulsby (1982), Singh *et al.*, (2011) and Jyothisree *et al.* (2013). Inclusion bodies of *A. marginale* conformed to those of workers (Atif *et al.*, 2012; Kakati, 2013).

According to the type of cattle, prevalence recorded was higher in crossbred (75.32%) compared to indigenous cattle (66.00%), the difference being highly significant ( $P < 0.01$ ). Crossbred animals were 1.57 times more susceptible to acquire tick-borne

haemoparasites than the indigenous cattle. These findings are in congruent with the results of several workers from India and abroad (Khan *et al.*, 2004; Nair *et al.*, 2013; Naik *et al.*, 2016). Atif *et al.*, (2012) in their work in Bangladesh reported significantly higher prevalence of tick-transmitted haemoparasitic diseases (Babesiosis, Anaplasmosis and Theileriosis) in crossbred as compared to indigenous cattle.

Findings of several workers concluded that indigenous breeds exhibit a high level of resistance to ticks and tick-transmitted diseases. According to Radostits *et al.*, (2000), crossbred cattle populations were more predisposed than indigenous cattle because of natural resistance and endemic stability between host-parasite relationships. Chaudhri *et al.*, (2013) also reported higher prevalence of *B. bigemina* (3.89%) in crossbred cows than in indigenous ones (1.53%), thus agreeing to our findings.

### **Sex wise prevalence of haemoparasites in cattle**

The female animals were found to have overall higher (74.02%) infection rate of haemoparasites than the male (58.22%) counterparts, the difference was found to be highly significant ( $P < 0.01$ ), as shown in Table-2. Prevalence of haemoparasite infection was recorded highest in females of Kokrajhar district (87.82%) and least in Baksa (67.81%),

The findings of the present work are in congruent with that of several workers (Rajput *et al.*, 2005; Kakati, 2013; Naik *et al.*, 2016). The possible reason for higher prevalence in female animals might be due to examination of more number of female cattle, hormonal disturbances and immunosuppression in advanced pregnancy and or lactation in high producing females.



### Age wise prevalence of haemoparasites in cattle

In the present study, the adult cattle (> 3 years) recorded highest infection of haemoparasites (80.42%) followed by young (68.11%) and calves (54.85%) which was highly significant ( $P < 0.01$ ), (Table-3) thus agreeing to reports of Ruprah (1985), Ananda et al (2009), Mohanta *et al.*, (2011), Kakati (2013) and Naik *et al.*, (2016). Adult cattle were 3.58 times more susceptible than calves to haemoparasitic infection. The lower prevalence in young animals compared to adults can be attributed to the restricted grazing of young animals which tends to reduce their chance of contact with the vectors of these diseases. It is assumed that aged animals are more susceptible to blood protozoan diseases than the younger animals due to inverse age resistance (Urquhart *et al.*, 1996).

In conclusion, the present study conducted for the first time in Indo- Bhutan border districts of Assam showed haemoparasite infection due to *T. orientalis*, *B. bigemina* and *A. marginale* in the cattle population and are considered to be endemic for the haemoparasites.

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