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Prevalence status of bovine tropical theileriosis in northern India

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Abstract

Considering the economic importance and increasing reports of bovine tropical Theileriosis (BTT) caused by *Theileria annulata*, from different agroclimatic regions of India, prevalence study was conducted to determine the status of BTT host-wise, age-wise and season wise in four northern states from July 2020 to June 2021 using microscopic thin blood smear examination.

Out of 544 blood samples (383 from cattle and 161 from buffaloes) collected from Uttarakhand, Haryana, Uttar Pradesh and Rajasthan, 86 (15.80%) were found positive for BTT (19.00% cattle and 07.45% buffaloes). State-wise prevalence was recorded maximum (17.37%) in Uttarakhand, followed by 16.52% in Uttar Pradesh, 15.58% in Haryana and minimum (9.67%) in Rajasthan. Age-wise prevalence was found maximum (20.45%) in animals of > 3 years of age (25.13% in cattle and 09.87% in buffaloes), followed by 12.84% in 1-3 year age group (15.50% in cattle and 06.00% in buffaloes) and minimum (8.91%) in animals < 1 year of age (11.26% in cattle and 03.33% in buffaloes). Seasonal prevalence was highest (18.18%) during summer followed by rainy (15.15%) and lowest (10.75%) during winter season. The present study concludes that bovines of northern India are highly susceptible for BTT, and proper diagnosis by veterinarians can minimize economic losses for farmers.

Keywords: Prevalence, bovine tropical theileriosis, blood smear examination, India

Introduction

Agriculture and allied sciences contribute as an integral part of Indian economy and around 54.6% of total Indian population is actively engaged in agriculture and other agro-based practices for livelihood (Census, 2011) [11]. As per the provisional estimates released by Central Statistics Office the agriculture and allied sector contribute about 17.3% of India's Gross Value Added (Central Statistics office, 2016-17) [8]. According to the 20th livestock census, total livestock population of India is 535.78 million, including 192.49 million cattle and 109.85 million buffaloes (www.dahd.nic.in) [9]. In order to keep the livestock population disease free and maintain productivity, there is a need of humungous efforts in terms of accurate disease diagnosis and strategies managemental practices.

According to Annual Report of National Institute of Veterinary Epidemiology and Disease Informatics (2019) [10], seven parasitic diseases viz. Coccidiosis, Babesiosis, Schistosomiasis, Trypanosomiasis, Fascioliosis, Theileriosis and Anaplasmosis are listed among the top 10 diseases responsible for major economic losses to livestock industry. The tick-borne haemoprotozoan parasites especially *Theileria* is one of the major causes of the economic losses (Devendra, 1995) [3].

Theileria is an obligate intracellular haemoprotozoan parasite which infects a wide range of hosts, including domestic and wild animals. It comes under Subkingdom Protozoa, Phylum Apicomplexa, Class Sporozoa, Subclass Piroplasmia, Order Piroplasmida and Family Theileriidae (Soulsby, 1982) [26]. The outcome of theileriosis may range from clinically inapparent to rapidly fatal disease (Taylor *et al.*, 2007) [27]. The common clinical signs indicative of *T. annulata* infection are pyrexia, enlargement of superficial pre- scapular lymph nodes, depressed rumination, excessive salivation, cachexia, nasal and ocular discharge (Osman and Al-Gaabary, 2007) [22]. The leucoproliferative and leucodestructive phases of bovine tropical theileriosis (BTT) can lead to acute anaemia and icterus with high morbidity and decreased production in the affected animals including domestic cattle and

Asian buffaloes (Ruprah, 1985) [24]. Adverse effects of the disease are more prominent in cross bred animals and young indigenous calves (Gautam, 1981) [6]. Mortality due to BTT varies from 90% in introduced exotic breeds to 5% or less in indigenous breeds (Neitz, 1957) [21].

Prevalence of theileriosis has been reported from various parts of India, including Uttarakhand (Rialch *et al.*, 2013; Arun Raj *et al.*, 2014; Nagar, 2018) [23, 2, 18]. The large-scale cross breeding programmes in India, aiming the up gradation of indigenous breeds, eventually led to increase in milk production and decreased resistance to theileriosis (Anandan, 1992) [1]. The inefficient tick control practices made it hard to rear exotic and crossbred cattle free from infection (Mehta *et al.* 1988) [15].

The economic losses of theileriosis have been estimated by various workers worldwide. Memon *et al.* (2017) [16] stated that theileriosis is one of the major causes of economic losses in terms of reduced quality and quantity of milk and meat for livestock holders. Narladkar (2018) [20] estimated a total loss of US \$ 1295 million due to tropical theileriosis in India as on October 2017. Hurtado and Rios (2018) [12] estimated a total economic loss of US \$ 14 to 19 billion due to ticks and tick-borne diseases. However, Nagar (2018) [18] recorded a total economic loss of Rs. 789.85 per animal due to BTT in Uttarakhand State.

In view of economic importance and wide prevalence of *T. annulata*, the present study was carried out to elucidate the prevalence of bovine tropical theileriosis in different states of Northern India.

Materials and Methods

Study area

The study was conducted to determine the prevalence of bovine tropical theileriosis in various states of Northern India over a period of one year (July, 2020 to June, 2021). Various states of Northern India namely Uttarakhand, Uttar Pradesh, Haryana and Rajasthan were selected to establish the prevalence of the disease.

Selection of animals

A total of 544 blood samples, comprising 383 blood samples of cattle and 161 samples from buffaloes, were screened in the present study. The data on prevalence of *T. annulata* infection in animals were generated according to state wise, host-wise, age-wise and season wise viz. summer (March to June), rainy (July to October) and winter (November to February).

Collection of blood samples

Blood samples were collected by jugular vein-puncture in EDTA-containing vacutainers randomly from both apparently healthy and clinically suspected cattle and buffaloes (n = 544) of various states namely Uttarakhand, Uttar Pradesh, Haryana and Rajasthan. Peripheral thin blood smear of each sample was prepared immediately and fixed with absolute methanol at the time of blood collection from states other than Uttarakhand. All blood samples and fixed blood smears were brought to the Department of Veterinary Parasitology, College of Veterinary and Animal Sciences, GBPUAT, Pantnagar for screening of theileriosis.

Processing of blood samples

Thin blood smear was prepared from each blood sample using grease free microscopic glass slides. A small pin head

size blood drop was placed on one end of slide and thin blood smear made using a spreader slide placed at an angle of 45 degree on the 1st glass slide. The spreader slide was pushed gently in forward direction to obtain a tongue shaped thin blood smear (Soulsby, 1982) [26]. The prepared blood smear was labelled and air dried for further processing.

Staining and microscopic examination of blood smears

After air drying, the blood smear was fixed using 100% methanol for 1 min and then stained with Giemsa stain of 1:20 dilution and kept for 30-40 minutes. The working solution of Giemsa stain was freshly prepared by mixing 1 part of stock solution with 19 parts of buffer solution at pH 7.2. After staining, the stained blood smear was washed in slow running water and air dried. The stained blood smear was, then, examined microscopically under oil immersion objective lens (X100 magnification) for the presence or absence of *Theileria* parasites.

Identification of *T. annulata*

The piroplasms and schizont stages of *T. annulata* were identified as per their morphological features (Soulsby, 1982) [26]. Thus, the prevalence data was generated.

Calculation of percent prevalence of *T. annulata*

Percent prevalence of *T. annulata* was determined by using formula as given by Thrusfield (1995) [28].

$$P = d/n \times 100$$

Where;

- P = Prevalence,
- d = Number of animals (Cattle/buffaloes) positive for Bovine Tropical
- Theileriosis,
- n = Total number of animals (Cattle/buffaloes) sampled

Statistical analysis

The epidemiological data was analysed using Pearson's chi-square (χ^2) test to assess whether the various groups involved into analysis share any statistically significant association at 5% level of significance ($p < 0.05$) (Wasihun and Doda 2013) [31].

Results

Overall prevalence of bovine tropical theileriosis in northern India

Samples were screened using microscopic thin blood smear examination stained with Giemsa stain for the presence of piroplasms (Figure 1) and Koch's blue bodies (KBB) (Figure 2) of *Theileria annulata*. Out of 544 blood samples, 213, 154, 115 and 62 blood samples from cattle and buffaloes suspected for theileriosis were examined from Uttarakhand, Haryana, Uttar Pradesh and Rajasthan state, respectively. Among 544 blood samples examined, a total of eighty-six blood samples were found positive for bovine tropical theileriosis, resulting in an overall prevalence of 15.80% in large ruminants of Northern India (Table 2, Figure 4). Among the infected blood samples, KBB were observed in 14 (2.57%) blood samples, piroplasms in 62 (11.39%) and both KBB and piroplasm in 10 (1.83%) blood samples. The results are presented in Table 1 and Figure 3.

A total of 383 blood samples from cattle were screened using Giemsa-stained thin blood smear examination for the presence of piroplasms and KBB. Out of all the examined samples, 74 (19.32%) blood samples from cattle were found positive for theileriosis. Of which, 11 (2.87%), 55 (14.37%) and 8 (2.08%) blood samples were found positive for KBB, piroplasms and both KBB and piroplasms, respectively. However, out of 161 blood samples of buffaloes, 12 (7.45%) blood samples were found positive for theileriosis. Of which, 3 (1.86%), 7 (4.34%) and 2 (1.24%) blood samples were found positive for KBB, piroplasms and both KBB and piroplasms, respectively (Table 1, Figure 3). State-wise prevalence of bovine tropical theileriosis was found maximum (17.37%) in Uttarakhand, followed by Uttar Pradesh (16.52%), Haryana (15.58%) and minimum (9.67%) in Rajasthan. The overall percent prevalence of bovine tropical theileriosis in different states of Northern India is presented in Table 2 and Figure 4.

Host-wise prevalence of bovine tropical theileriosis in different states of northern India

Host-wise prevalence revealed significantly ($p < 0.05$) higher (19.32%) infection in cattle as compared to buffaloes (7.45%) in northern India. Maximum (20.83%) infection of *T. annulata* was recorded in cattle of Uttarakhand, followed by Uttar Pradesh (20.25%), Haryana (20.21%) and minimum (9.52%) in Rajasthan state. However, in buffaloes, it was observed maximum (10.00%) in Rajasthan, followed by Uttar Pradesh (08.33%), Haryana (08.33%) and minimum (04.44%) in Uttarakhand state (Table 2, Figure 4).

Age-wise prevalence of bovine tropical theileriosis in different states of northern India

Significantly ($p < 0.05$) higher infection rate was recorded in animals above 3 years of age, followed by those between 1-3 years of age and less than 1 year of age. Overall age-wise prevalence of bovine tropical theileriosis was found maximum (20.45%) in animals above 3 years of age, followed by those between 1-3 years of age (12.84%) and minimum (8.91%) in animals less than 1 year of age (Table 3, Fig- 5). Among cattle, overall maximum (25.13%) prevalence was seen in cattle above 3 years of age, followed by those between 1-3 years of age (15.50%) and minimum (11.26%) in cattle less than 1 year of age. Among buffaloes, overall maximum (09.87%) prevalence was seen in buffaloes above 3 years of age, followed by those between 1-3 years of age (6.0%) and minimum (03.33%) in buffaloes less than 1 year of age.

Season-wise prevalence of bovine tropical theileriosis in different states of northern India

Significant difference ($p < 0.05$) was recorded in the seasonal prevalence of bovine tropical theileriosis in different states of Northern India. Out of 544 examined blood samples, a total of 253, 198 and 93 blood samples were examined during summer, rainy and winter seasons, respectively. Seasonal percent prevalence of bovine tropical theileriosis was recorded maximum (18.18%) in summer season followed by rainy (15.15%) season and minimum (10.75%) during winter season (Table- 4, Fig- 6).

Table 1: Overall percent prevalence of bovine tropical theileriosis in northern India

Animal	NE	KBB (%)	Piroplasm (%)	Both (%)	Total (%)
Cattle	383	11 (2.87)	55(14.37)	8 (2.08)	74 (19.32)
Buffaloes	161	3 (1.86)	7(4.34)	2 (1.24)	12 (7.45)
Overall	544	14 (2.57)	62(11.39)	10 (1.83)	86 (15.80)

Table 2: Overall percent prevalence of bovine tropical theileriosis in different states of northern India

States	% Prevalence (Cattle)	% Prevalence (Buffaloes)	Overall % Prevalence (95% CI) ^a
Uttarakhand	20.83	4.44	17.37 (12.84-23.06)
Haryana	20.21	8.33	15.58 (10.64-22.20)
Uttar Pradesh	20.25	8.33	16.52 (10.76-24.45)
Rajasthan	9.52	10.00	9.67 (4.17-19.89)
Overall	19.32	7.45	15.80

^a Confidence interval ($p < 0.05$)

Table 3: Overall age-wise prevalence of bovine tropical theileriosis in different states of northern India

Host	Age-wise percent prevalence			Total (95% CI) ^a
	Less than 1 year (95% CI) ^a	1-3 years (95% CI) ^a	More than 3 years (95% CI) ^a	
Cattle	11.26 (5.57-20.94)	15.5 (10.19-22.81)	25.13 (19.39-31.91)	19.32 (15.67-23.58)
Buffaloes	3.33 (<0.1-18.09)	6 (1.44-16.84)	9.87 (4.86-18.53)	7.45 (4.20-12.69)
Overall	8.91 (4.57-16.26)	12.84 (8.66-18.60)	20.45 (16.01-25.75)	15.80 (12.97-19.12)

^a Confidence interval ($p < 0.05$)

Table 4: Seasonal prevalence of bovine tropical theileriosis in northern India

Season	No. of samples examined	No. of samples positive	Percent Prevalence (95% CI) ^a
Rainy	198	30	15.15 (10.78-20.85)
Winter	93	10	10.75 (5.77-18.85)
Summer	253	46	18.18 (13.89-23.42)
Overall	544	86	15.80

^a Confidence interval ($p < 0.05$)

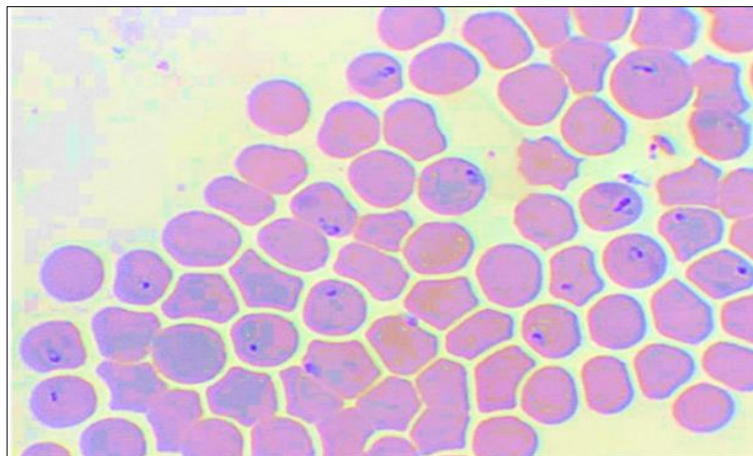


Fig 1: Giemsa-stained blood smear showing piroplasms of *T. annulata* in red blood cells (X1000)

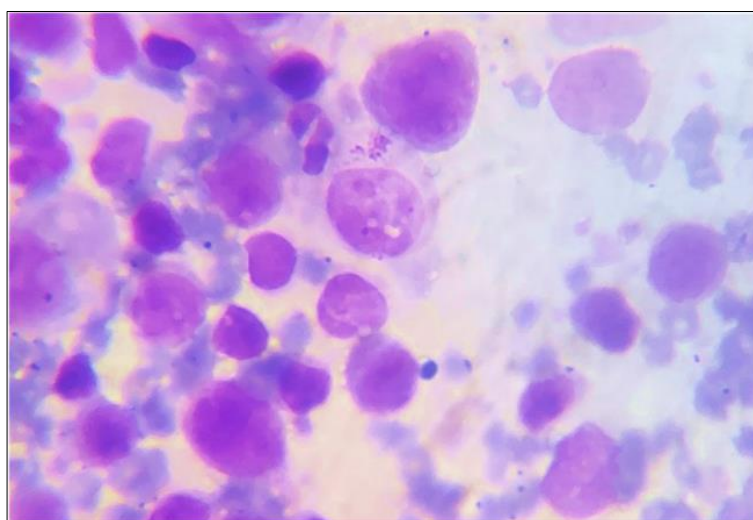


Fig 2: Giemsa-stained blood smear showing Koch's Blue Bodies (schizonts) in lymphocytes (X1000)

Discussion

Overall prevalence of bovine tropical theileriosis in northern India

In the present study, out of 544 blood samples examined, a total of 86 blood samples were found positive for bovine tropical theileriosis, resulting in an overall prevalence of 15.80% in large ruminants of Northern India. Various authors have reported the prevalence of from different agro-climatic zones of India (Ganguly *et al.*, 2017; Khorajiya *et al.*, 2017; Khatoon *et al.*, 2021; Sharma *et al.*, 2021) [5, 14, 13, 25]. The overall prevalence of bovine tropical theileriosis recorded from Uttarakhand was found almost coinciding with Sharma *et al.* (2021) [25]. They observed an overall prevalence of 20.77% from six different districts of Uttarakhand. The slight differences in the results might be due to variations in agro-climatic conditions and the managemental practices followed by the farmers of the selected areas. The prevalence rate of bovine tropical theileriosis recorded from Haryana during the present study was found in accordance with the findings of Ganguly *et al.* (2017) [5]. Khorajiya *et al.* (2017) [14] recorded a higher (35.71%) prevalence rate of *T. annulata* infection from Uttar Pradesh as compared to the results of the present study. A higher (67.22%) prevalence as compared to the present study was reported from Rajasthan by Khatoon *et al.* (2021) [13]. The differences in the results might be due to variations in the geo-climatic conditions and the density and

distribution of reservoir host-vector population in the experimental areas.

Host-wise prevalence of bovine tropical theileriosis in different states of northern India

The present study recorded significantly ($p < 0.05$) higher prevalence of bovine tropical theileriosis among cattle as compared to buffaloes. Similar results have been observed by various workers (Arunraj, 2014; Khorajiya *et al.*, 2017; Sharma *et al.* 2021) [2, 14, 25]. Higher prevalence of bovine tropical theileriosis in cattle may be due to thinner skin and dry habitat of cattle which makes them more susceptible for tick infestation as compared to buffaloes. Also, in case of buffaloes, dropping of ticks may occur during wallowing, leading to lower rate of tick infestation as compared to cattle.

Age-wise prevalence of bovine tropical theileriosis in different states of northern India

In the present study, significantly ($p < 0.05$) higher prevalence of bovine tropical theileriosis was observed in animals above 3 years of age, followed by those between 1-3 years of age and less than 1 year of age. These findings are in agreement with earlier reports from various workers across India (Arunraj, 2014; Naik *et al.* 2016; Sharma *et al.*, 2021) [2, 19, 25]. It is already documented that some physiological factors like pregnancy, lactation and oestrus may cause temporary suppression in the immunity of aged

animals making them highly susceptible for getting an infection (Durrani, 2008) ^[4]. Calves are more resistant to theileriosis as compared to the older cattle (Utech and Wharton, 1982) ^[29]. Also, young animals may acquire passive immunity against schizonts, sporozoites and piroplasmids of *Theileria* sp. via the colostrum of immune dams (Morzaria *et al.*, 1988) ^[17].

Season-wise prevalence of bovine tropical theileriosis in different states of northern India: The results of seasonal

prevalence recorded in the present study are in accordance with the previous reports of Rialch *et al.* (2013), Arunraj (2014), Nagar (2018), Sharma *et al.* (2021) ^[23, 2, 18, 25]. Highest prevalence of BTT was recorded during summer season as hot and dry climatic conditions might favour the development of *T. annulata* sporozoites in the suitable tick vector (Haque *et al.*, 2010) ^[7]. Also, high temperature and humid conditions are considered suitable for the survival and breeding of ticks, leading to the abundance of ticks during such climatic conditions (Velusamy *et al.*, 2014) ^[30].

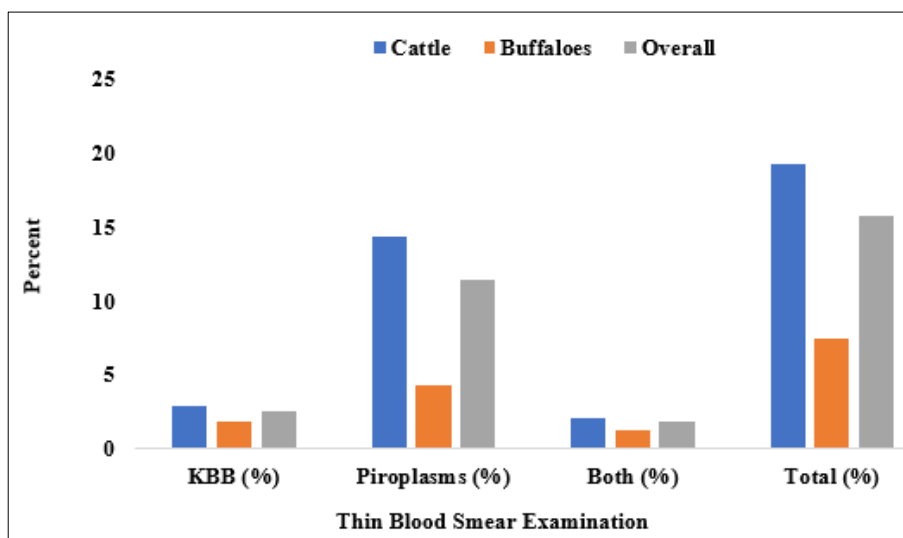


Fig 3: Overall percent prevalence of bovine tropical theileriosis in Northern India

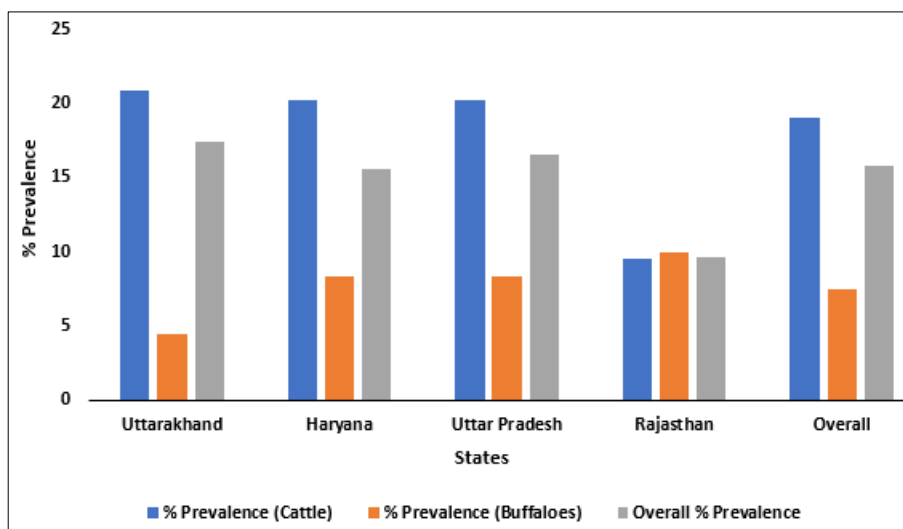


Fig 4: Overall percent prevalence of bovine tropical theileriosis in different states of Northern India

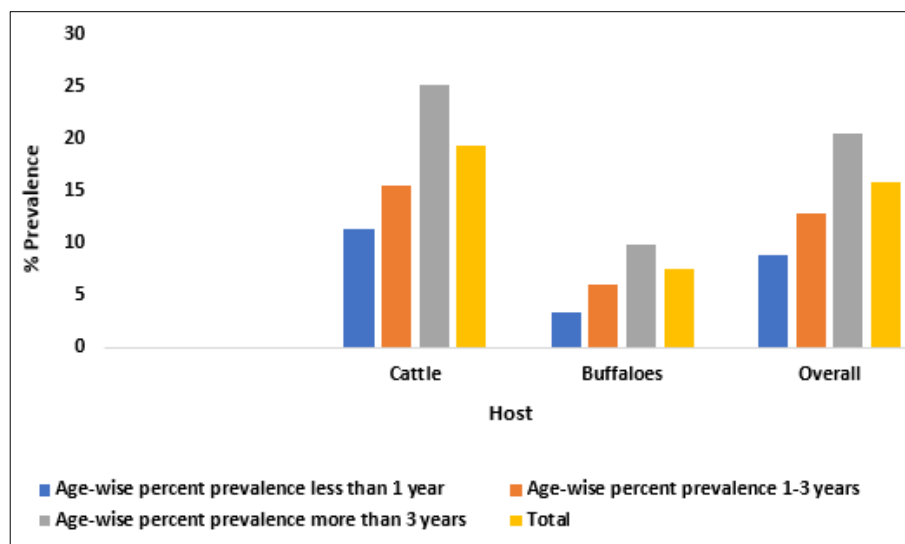


Fig 5: Overall age-wise prevalence of bovine tropical theileriosis in different states of Northern India

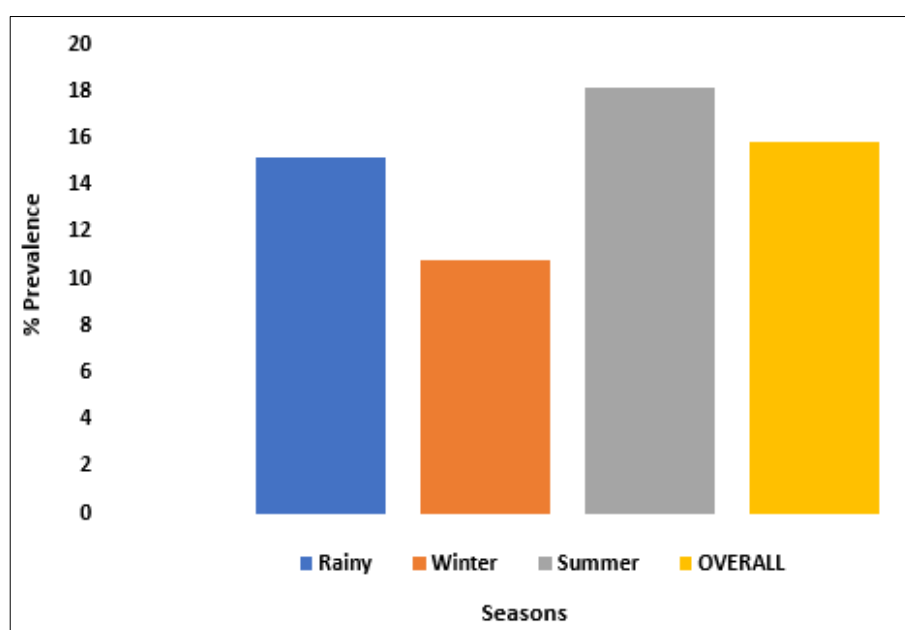


Fig 6: Seasonal prevalence of bovine tropical theileriosis in Northern India

Conclusion

Bovine tropical theileriosis, caused by *Theileria annulata*, is one of the major and commonly found haemoprotozoan infection in cattle and buffaloes responsible for huge economic losses in terms of qualitative and quantitative deterioration in milk, poor growth rate and even, mortality. As per the findings of present study, it can be concluded that bovines of Northern India are highly susceptible for bovine tropical theileriosis responsible for huge economic losses. The prevalence data may be used for formulating effective control strategies against the disease in Northern India. As the disease is fatal in nature, it is suggested that farmers should contact veterinarians for proper diagnosis and management of *T. annulata* infection in bovines to combat the economic losses.

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