Molecular Prevalence and Host Related Epidemiological Factors of Bovine Tropical Theileriosis in Semi-Arid Zone of Northern Plains of India

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ABSTRACT

The study was conducted at VCC, DUVASU, Mathura, during the period of November, 2021 to October, 2022a total of 145 cattle and 155 buffaloes suspected to be affected with theileriosis were examined using PCR technique for identification of *T. annulata*. The overall prevalence of theileriosis in cattle and buffaloes was found 7.19% (n=) and 3.32% (n=), respectively. The prevalence in suspected cattle and buffaloes was found 78.62% and 49.23%. Highest prevalence in cattle (91.66%) and buffalo (64.28%) was recorded during the month of April and lowest prevalence was recorded during October month in cattle (42.85%) and December month in buffaloes (10%). Prevalence of theileriosis was recorded higher in female cattle (82.30%) as compared to males (46.66%) however, no variation on the basis of sex was observed in buffaloes. Significant increase in the prevalence was observed in cattle ($\chi2 = 6.24$, p = 0.044) and buffaloes ($\chi2 = 12.856$, p = 0.002) on the basis of age. Significant increase in the prevalence of theileriosis was observed in different breeds of cattle ($\chi2=8.913$, p=0.030) while, in buffaloes ($\chi2 = 0.039$, p = 0.844), no significant variation in the prevalence was recorded on the basis of breed. Significant variation was observed in different parity of buffaloes ($\chi2 = 12.361$, p = 0.006), highest prevalence were recorded in buffaloes have > 3 parity, but no variation is observed in different parity of cattle ($\chi2 = 4.789$ p = 0.091). No variation was observed in the prevalence of theileriosis in cattle ($\chi2 = 3.817$, p = 0.051) and buffaloes ($\chi2 = 0.460$, p = 0.498) on the basis of pregnancy.

HIGHLIGHTS

⊙ Molecular prevalence and host related epidemiological factors of theileriosis.
⊙ The overall prevalence in cattle and buffaloes was found 7.19% and 3.32%.
⊙ Study will ease epidemiological investigation on theileriosis.

Keywords: Epidemiological factors, Theileriosis, Variations, Pregnancy

The livestock plays an important role in the economy of farmers but parasitic infection on animals is a major problem that affects milk production and therefore affects the economy of the farmers, while haemoparasites affect the host's immune system and damage red blood cells, leading to various severities of anaemia. The direct result of parasitism in dairy cows is lower milk production due to morbidity and mortality of parasitized animals. Tick-borne diseases (TBDs) cause major economic losses and affect many domestic animals, mainly cattle and sheep, in tropical and subtropical regions. Tropical theileriosis is a TBD caused by a protozoon called *Theileria annulata* transmitted by several tick species of the genus *Hyalomma*. Theileria belongs to Family Theileriidae, Order Piroplasmida, Subclass Piroplasma, Phylum

Apicomplexan obligate intracellular protozoan parasites that infect both wild and domestic bovidae throughout the world. Some species also infect small ruminants. *Theileria spp.* are transmitted by ixodid ticks with complex life cycles in both vertebrate and invertebrate hosts. The two most pathogenic and economically important species are *T. parva* and *T. annulata* (Pal and Chakravarty, 2020). In India economic loss due to blood parasitic diseases in animals has been estimated to about USD 498.7 million per annum, The estimated economic loss due to tropical theileriosis is US$ 1,295 million (8,426.7 crore) annually (Rajendran and Ray, 2014; Narladkar, 2018).

Mathura district situated along the banks of the river Yamuna is a district of Uttar Pradesh state of north-central India, located at 27.28°N 77.41°E, has an average elevation of 174 metres (570 feet). The climate of Mathura is tropical extreme with very hot summers with temperatures rising beyond 44 °C. Mathura and its surrounding areas animals are more prone for exposure to tick vector; because of the fact that in this area there is having higher vector density due to its agro climatic condition, therefore, the animals might be getting acute infection of *Theileria anulata*. The present investigation was conducted with a comprehensive plan to study the molecular prevalence and host related epidemiological factors of bovine tropical theileriosis in semi-arid zone of northern plains of India.

MATERIALS AND METHODS

The study was performed at TVCC, DUVASU, Mathura, from November 2021 to October 2022. Animals came with history of suffering with fever, inappetance/anorexia, loss of production, anaemia and loss of body condition was selected for study. Total number of cattle and buffaloes coming to the TVCC for different ailments and number of cattle and buffaloes suspected, were recorded on monthly basis. The epidemiological data was also recorded on the basis of month, sex, age, breed, parity and pregnancy status. Clinico-epidemiological studies were done by assessing frequency distribution of various clinical signs in each and every positive cases and data related to month, sex, age, breed, parity, pregnancy status, in each and every suspected case of theileriosis in cattle and buffaloes.

STATISTICAL ANALYSIS

Statistical analysis between various host related factor was analysed by Chi-square test. P values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

Clinico-epidemiological studies were done in the *Theileria* positive animals which shown positivity of *T. anulata* on the basis of PCR. The overall prevalence of theileriosis in cattle and buffaloes at TVCC, DUVASU, Mathura, during the period from November (2021) to October (2022) was found to be 7.19% and 3.32% respectively, however, the prevalence of theileriosis in suspected cattle and buffaloes during the period was found to be 78.62% and 49.23% (Table 1, 2).

Table 1: Percent prevalence of Theileriosis in Cattle at TVCC, DUVASU, Mathura in different months

Sl. No.	Month	Total no of Animals reported	No of cases suspected	Positive no of cases	Prevalence (%)	Overall prevalence (%)
1	November (2021)	105	09	05	55.55	4.76
2	December (2021)	110	05	03	60	2.72
3	January (2022)	95	4	02	50	2.10
4	February (2022)	141	04	02	50	1.41
5	March (2022)	170	10	07	70	4.11
6	April (2022)	121	12	11	91.66	9.09
7	May (2022)	125	09	6	66.66	4.8
8	June (2022)	139	12	9	75	6.47
9	July (2022)	128	35	32	91.42	25
10	August (2022)	122	25	22	88	18.03
11	September (2022)	206	13	11	84.61	5.33
12	October (2022)	122	07	03	42.85	2.45
Total			1584	145	114	78.62

Table 2: Percent prevalence of theileriosis in Buffaloes at TVCC, DUVASU, Mathura in different month

| Sl. No.	Month	Total no of Animals reported	No of cases suspected	Positive no of cases	Prevalence (%)	Overall prevalence (%)
1	November (2021)	181	8	04	50.00	2.20
2	December(2021)	190	10	01	10	0.52
3	January (2022)	112	6	01	16.66	0.89
4	February (2022)	150	7	02	28.57	1.33
5	March (2022)	195	5	02	40	1.02
6	April (2022)	178	14	09	64.28	5.05
7	May (2022)	117	19	11	57.89	9.40
8	June (2022)	167	19	10	52.63	5.98
9	July (2022)	218	18	09	50	4.13
10	August (2022)	254	18	11	61.11	4.33
11	September (2022)	323	17	09	52.94	2.78
12	October (2022)	200	14	07	50	3.50
Total		**2285**	**155**	**76**	**49.23**	**3.32**

Prevalence study in different months

Highest prevalence of theileriosis in cattle and buffalowas recorded during the month of April and lowest prevalence was recorded during October month in cattle and December month in buffaloes (Fig. 1a, 1b).
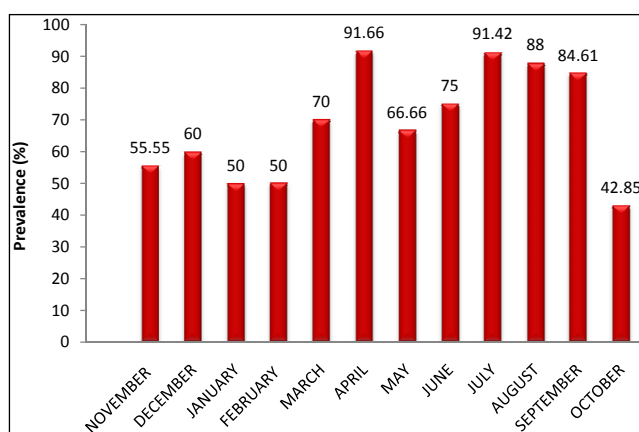
Fig. 1a: Showing percent prevalence in different months in Cattle

In the present study theileriosis in cattle and buffaloes were found to be prevalent throughout the year of study and increase in summer and wet months of monsoon and post monsoon season could be due to the abundance of vectors and the prevalence was less in winter months of year when there is least presence of vector. The findings of the present investigation regarding month wise prevalence are simulates with earlier findings that higher prevalence of

theileriosis observed in summer, monsoon, post-monsoon and least in the winter months/season of the year (Selim *et al*., 2022; Maharana *et al*., 2016; Velusamy *et al*., 2014). The month/season wise variations in the prevalence pattern of *T. annulata* infections in cattle and buffaloes could be due to the increase in the vector population in considerable number during summer, rainy and post-rainy months than the winter months of the year (Waskel and Gaur, 2015).There was significant difference in prevalence of theileriosis in male and female cattle and recorded 46.66% and 82.30% respectively. The prevalence of theileriosis in female buffaloes was recorded 50% while none of male buffalo reported was found positive from theileriosis.
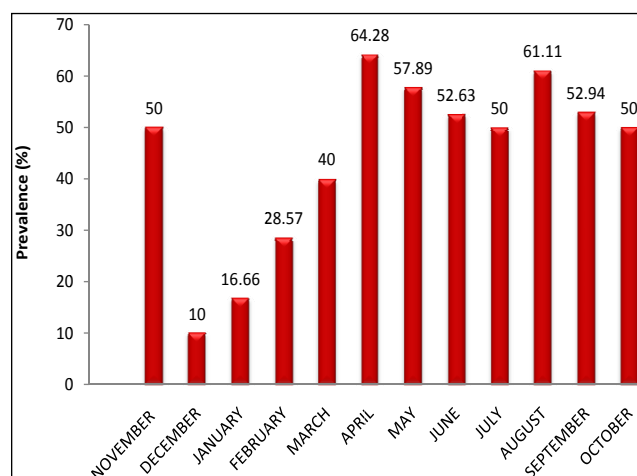
Fig. 1b: Showing percent prevalence in different months in Buffaloes

Prevalence of theileriosis in Cattle and buffaloes according to various host related factor

Sex wise

In the present study total 15 male cattle and 03 male buffaloes was suspected for theileriosis out of which 07 male cattle was found positive with a prevalence of 46.66% and none of male buffaloes was found positive for theileriosis and 130 female cattle and 152 female buffaloes was suspected for theileriosis out of which 107 cattle and 76 buffaloes was found positive with prevalence of 82.30% and 50% respectively. There is significant higher prevalence (p<0.05) of theileriosis recorded in female cattle in comparison to male. However no variation on the basis of sex was observed in the prevalence of theileriosis in buffaloes (Table 3, 4). The present investigation also in line that higher prevalence of theileriosis in females than males, as a potential risk factor for tropical theileriosis in the bovine population (Farooqi *et al*., 2017; Parveen *et al*.,

2021). The higher prevalence rate of tropical theileriosis in females is due to more hormonal fluctuation, weaken/disturbed immune response during the gestation or lactation period, and carrying more ticks that make them prone to tropical theileriosis (Parveen *et al*., 2021). Therefore, female cattle need more attention to reduce the risk of being infected with *T. annulata.*

Age wise and Parity

The highest prevalence was found in cattle and buffaloes of >5 years age, followed by 2-5 years of age and lowest prevalence was recorded in age <2 years (Table 3, 4). There was significant variation (p<0.05) observed in the prevalence of cattle and buffaloes on the basis of age. Therefore, from present study it can be said that the prevalence of theileriosis in cattle and buffaloes increases with age. The observations recorded regarding sex wise prevalence in the present investigation corroborates with previous report of higher prevalence in females (Selim *et*

Table 3: Percent prevalence of theileriosis in Cattle according to various host related factor

Host related factors	No of examined (145)	No of positives (114)	Prevalence (%) (78.62)	Chi-square value
Sex				
Male	15	7	46.66	$\chi 2$ = 10.163 P=0.001
Female	130	107	82.30	
Age, years				
< 2	29	18	62.06	
2-5	45	36	80	$\chi 2$ = 6.241, P = 0.044
> 5	71	60	84.50	
Breed				
Haryana	21	15	71.42	
Sahiwal	25	21	84	
Cross Breed	52	50	96.15	$\chi 2$ = 8.913, P = 0.030
ND (Cattle)	32	28	87.5	
Parity				
Zero	18	14	77.77	
1	11	8	72.72	
2 to 3 times	37	32	86.49	$\chi 2$ = 4.789, P = 0.091NS
More than 3 times	64	60	93.75	
Pregnancy status				
Pregnant	90	78	86.73	$\chi 2$ = 3.817, P = 0.051NS
Non-pregnant	40	29	72.50	

*NS= Shows no significance (P > 0.05).

Table 4: Percent prevalence of Theileriosis in buffaloes according to various host related factor

Host related factors	No of examined (155)	No of positives (76)	Prevalence (%) (49.03)	Chi-square value
Sex				$\chi2=2.943$
Male	03	00	00	P=0.086
Female	152	76	50	NS
Age, years				
< 2	15	02	13.33	$\chi2=12.856$
2-5	45	18	40.00	
> 5	95	56	58.95	P=0.002
Breed				
Murrah	62	31	50	$\chi2=0.039$
				P=0.844
Non descript	93	45	48.38	
				NS
Parity				
Zero	27	6	22.22	
1	31	14	45.16	
2 to 3 times	34	19	55.88	$\chi2=12.361$
More than 3 times	60	37	61.66	P=0.006
Pregnancy status				
Pregnant	98	51	52.04	$\chi2=0.460$
				P=0.498
Non pregnant	54	25	46.29	
				NS

* NS= Shows no significance (P> 0.05).

al., 2022; Ullah *et al*., 2021; Zeb *et al*., 2020). In cattle, no significant variations in the prevalence of theileriosis were observed. However, in buffaloes significantly higher prevalence was recorded in >3 parity, followed by 2-3 parity and followed by 1 parity. Lowest prevalence was recorded in zero parity (Table 3, 4). There is significant variation (p<0.05) recorded in the prevalence of theileriosis in different parity of buffaloes but no variation is observed in the prevalence of theileriosis in different parity of cattle. Therefore, from present study it can be said that the prevalence of theileriosis in cattle not varies with the parity but in buffaloes varies with the parity. The possible reason regarding prevalence of parity was observed that more number of cattle calves and heifers were found to be positive for theileriosis in comparison to buffaloes that could be the reason that, there was no significant variation in the prevalence of theileriosis according to parity was observed in cattle. The present findings could not be explained because of paucity of literature.

Although parity is related with the age therefore, higher prevalence with the increase in the parity of the buffaloes was observed, it might be due to the stress factors like lactation and pregnancy makes the buffaloes predisposed for the infection apart from exposure to the vector which is primary source for the transmission of the infection.

Prevalence study according to Breed

In cross breed cattle highest prevalence of theileriosis was observed, followed by in Non-descript breed, Sahiwal breed and least in Haryana breed. However, in buffaloes, no significant variations in the prevalence were recorded among Murrah breed and ND buffalo (Table 3,4). There is significant variation (p<0.05) recorded in the prevalence of theileriosis in different breeds of cattle but no variation is observed in the prevalence of theileriosis in different breeds of buffaloes. Therefore, from present study it can be said that the prevalence of theileriosis in cattle varies in

different breed but in buffaloes does not vary in different breeds. The higher prevalence in cross bred cattle than other breeds of cattle under study has been observed by other previous researchers and findings of present investigation are in agreements with the earlier reports (Anand and Ross, 2021; Ullah *et al.*, 2021; Zeb *et al.*, 2020; Farooqi *et al.*, 2017). In indigenous cattle low prevalence of theileriosis might be due to low tick infestation, higher resistance to tick infestation, lower acute-phase protein responses controlled by macrophage cytokines, enzootic stability and in comparison to cross bred cattle.

Prevalence study according to Pregnancy

In the present study no significant variation (p>0.05) in the prevalence of theileriosis was recorded on the basis of pregnancy in both cattle and buffaloes (Table 3, 4). Prevalence of theileriosis in cattle and buffaloes do not vary between pregnant and non-pregnant. Although pregnancy acts as stress to the animals but it is also a fact that more care and good nutrition is given to the pregnant animals than non-pregnant ones which minimizes the stress in the pregnant animals. Therefore, no variations in the prevalence of theileriosis in present study could be recorded between pregnant and non-pregnant animals. Pregnant cows seem to be particularly susceptible to clinical disease and may abort or develop anaemia during late gestation through to two to four months after calving. (Chowdhury, 2017).

CONCLUSION

In the present study a total of 145 cattle and 155 buffaloes suspected to be affected from theileriosis, giemsa stained thin blood smear revealed the presence of *T. anulata* in 72 cattle and 31 buffaloes showing an efficacy of 49% and 20% respectively. Polymerase chain reaction (PCR) examination of same number of suspected cattle and buffaloes revealed, the presence of *T. anulata* in 114 cattle and 76 buffaloes, showing an efficacy of 78.62% and 49.03% respectively. Therefore, molecular diagnostic technique (PCR) was found to be of maximum efficacy followed by giemsa stained thin blood smear examination.

ETHICAL APPROVAL

All of the procedures of this experiment were approved by the committee of ethics for research of college of veterinary science and animal husbandry, DUVASU, Mathura, UP, India.

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