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The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(9): 2572-2574

www.thepharmajournal.com
Received: 04-07-2022
Accepted: 12-08-2022

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Prevalence study of bovine theileriosis in Satara and Solapur District of Maharashtra

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Abstract
103 blood samples from crossbred cattles were collected randomly from 21 farmers in Khandala, Phaltan, Lonand and Pandharpur taluka of Satara and Solapur district. Samples were subjected for to test smear examination and species specific PCR. Data related to the farm and farmer was collected and correlated with prevalence. Prevalence of *Theileria spp* was 6.79% (7/103) and 80.58% (83103) with microscopy and PCR respectively. Prevalence of *T. orientalis* was 75.72% (78/103) followed by *T. annulata* 2.92% (3/103), while 2/103 (1.94%) were mixed infections. Sensitivity and specificity of blood smear against PCR to detect *T. orientalis* was 9% and 100% respectively.

Keywords: Prevalence, theileriosis, Maharashtra, sensitivity, specificity, PCR

Introduction
Haemoprotozoan infections (theileriosis, babesiosis and anaplasmosis) are very common in tropical and subtropical regions and cause major economic losses to the livestock industry (Velusamy *et al.,* 2014) [11]. The global annual economic losses due to tick borne diseases where alone US$18.7 billion, while in India US$ 498.7 million/annum (Ghosh and Nagar 2014) [2]. In India the theileria species-wise analysis indicated a high prevalence of *T. annulata* 21% followed by *T. spp* 17%, *T. orientalis* 16% (krishnamoorthy P *et al.,* 2021) [7]. In Maharashtra the prevalence *T. annulata* varies from 6.5% to 29.5% and for *T. orientalis* 1.9% to 11.8% (Kolte *et al.,* 2017) [5]. Hence, the present work was carried out to study prevalence of *T. annulata* and *T. orientalis* in Satara and Solapur districts of western Maharashtra.

Materials and methods
103 animals (cattles) blood samples were randomly collected and analysed from farmers in Satara and Solapur districts of Western Maharashtra from September 2021 to February 2022 (6 months). Approximately 10 ml blood was collect aseptically from jugular vein in Ethylene Diamine Tetra Acetic Acid (EDTA) vials (4 ml) and plain vials (6 ml) respectively. The blood smears prepared on the spot without any coagulant were fixed using absolute methanol and stained with Giemsa stain (1: 10 ratio). The DNA was isolated from whole blood by using Favorgen Blood Genomic DNA Extraction Mini Kit (FavorPrep™). The PCR reaction was set up into 25 µl reaction containing 0.5µl of each forward and reverse primers, 12.5µl Mater Mix (dNTPs, $MgCl_2$, Dye and Taq DNA Polymerase), 10.5 µl of nuclease free water and 1µl of DNA template (Table 3).

Table 1: The reaction condition for *T. annulata*

Step	Temperature (˚C)	Time (min)	No. of Cycles
Initial denaturation	98	0.01	
Denaturation	98	0.10	35 cycles
Annealing	55	0.30	
Extension	72	1	
Final extension	72	5	

Table 2: The reaction condition for *T. orientalis*

Step	Temperature (˚C)	Time (mins)	No. of Cycles
Initial denaturation	98	0.01	
Denaturation	98	0.10	35 cycles
Annealing	58	0.30	
Extension	72	1	
Final extension	72	5	

Table 3: Primers used for detection of 16s rRNA gene

Organism	Target gene	Primer sequence 5'-3'	Product size (bp)	References
Theileria annulata	Tams1	Forward primer $^{5''}$GTAACCTTTAAAAACGT$^{3''}$ Reverse primer $^{3''}$GTTACGAACATGGGTTT$^{5''}$	721	Durrani, *et al.*, 2008 [1]
Theileria orientalis	MPSP	Forward Primer 5″CTTTGCCTAGGATACTTCCT3″ Reverse Primer 5″ACGGCAAGTGGTGAGAACT3″	776	Ota *et al*., 2009

The PCR amplicons were then subjected to 0.8% of agarose gel electrophoresis and run 100 mV for 20 min. Gel was examined under Gel documentation System (Bio Doc-It™ Imager, UVP).

Results and discussion

Prevalence of *Theileria spp* by blood smear examination

7/103 (6.79%) cattles were positive for *Theileria spp* by microscopic examination of blood smear *viz*. in 4/44 (9.09%) Satara district and 3/59 (5.08%) Solapur district (Table 4). Above findings were agreement with Shah *et al.*, (2020) [9] and Jayalakshmi *et al.*, (2019) [3] who reported prevalence of theileriosis 5.83% and 7.8% respectively. Lower prevalence observed may be due to winter season of study, higher tick population occurred during summer and rainy season, due to hot and humid environment favours tick multiplication (Krishnamurthy *et al.*, 2016) [6].

Prevalence of *Theileria annulata* by Polymerase Chain Reaction

3/103 (2.92%) animals were positive for *T. annulata* by PCR *viz*. 3/44 (6.81%) in Satara district (Table 4). Sahoo *et al.*, (2017) [8] reported that 7/34 (20.59%) apparently healthy lactating cow were *T. annulata* positive by PCR in Odisha. Kolte *et al.*, (2017) [5] reported overall prevalence of *T. annulata* (15.8%) in different agro-climatic zones of Maharashtra by PCR. They further reported that relatively lower prevalence *T. annulata* (6.5%) was reported in Scarcity Zone. Prevalence of *T. annulata* observed in this study is lower than the figures reported by researchers, however there is wide difference in the prevalence of the disease in different geographical areas and agro-climatic zones. Prevalence may differ during different seasons. Prevalence observed is similar to the reported values in nearby districts in Maharashtra.

Prevalence of *Theileria orientalis* by Polymerase Chain Reaction

78/103 (75.72%) animals were positive for *T. orientalis* by PCR *viz*. Satara district 32/44 (72.72%) and Solapur 46/59 (77.96%) (Table 4). Sharma *et al.*, (2019) [10] reported 52% molecular prevalence of *T. orientalis* in Vidarbha region of Maharashtra. 2/103 (1.94%) animals found positive by PCR for mix infection (Table 4) findings are agreement with Sahoo *et al.*, (2017) [8] who reported that 11 (32.35%) cows were positive for *Thieleria spp.*, while 7/30 (20.59%) *T. annulata* and 3/30 (8.82%) *T. orientalis* and 2.94% (solitary case) of mix infection.

Sensitivity and Specificity of Blood smear and against PCR

Sensitivity and specificity of blood smear against PCR to detect *Theileria orientalis* was 9% and 100% respectively while positive and negative predictive values were 100% and 26.04% respectively (Figure 1). Khatoon *et al.*, (2015) [14] reported that the sensitivity were 23.88% and specificity 90.47% of blood smear in animals compare to PCR.

Table 4: Result of microscopy and PCR for detection of *Theileria spp.* in bovine

Species	PCR	Microscopy
T. annulata	3	7
T. orientalis	78	
Both	2	
Negative	24	96

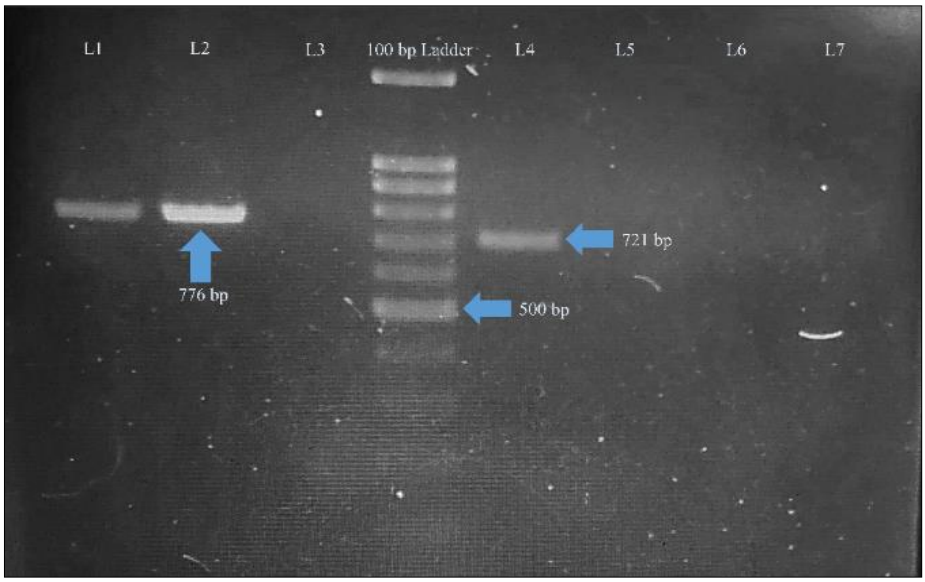

Fig 1: Agarose gel electrophoresis of Sample L1 and L2 (*T. orientalis*), Sample L4 (*T. annulata*).

Conclusion

Overall prevalence of *Theileria spp.* was 6.79% by blood smear examination while prevalence of *Theileria annulata* and *Theileria orientalis* was 2.92% and 75.72% respectively by PCR. *Theileria orientalis* was predominant infection found in this study without overt clinical signs. Sensitivity and specificity of blood smear against PCR to detect *Theileria orientalis* was 9% and 100% respectively. Blood smear can be useful for diagnosis of clinical theileriosis (high sensitivity) but cannot be used as a screening test (low sensitivity).

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