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Seasonal Prevalence of Ovine and Caprine Theileriosis in Tamil Nadu

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A B S T R A C T

Ovine and caprine theileriosis is most prevalent and drastic hemoprotozoan disease of sheep and goats. The current study was conducted to inspect the seasonal prevalence of ovine and caprine theileriosis from December 2016 to November 2017 in Tamil Nadu. For this purpose, total 780 blood samples were taken, 360 from sheep and 420 from goats in different seasons of Tamil Nadu. Out of 780 samples, 75.76 per cent (591/780) blood samples were positive for *Theileria* spp. by PCR. The season wise prevalence revealed that, the highest prevalence was observed in summer months followed by South West monsoon, North East monsoon and winter season.

Introduction

Theileriosis is an important hemoprotozoan disease of sheep and goats in tropical and subtropical regions caused by the different species of *Theileria* (Altay *et al.,* 2007). They are intracellular parasites that complete their life cycle in the mammalian hosts by successively utilizing lymphoid cells and erythrocytes. In malignant ovine theileriosis, the hosts exhibit severe clinical signs like high fever, anaemia, lymphnode enlargement, listlessness, anorexia, emaciation, intermittent diarrhoea or constipation and may lead to death in peracute form of the disease (Tageldin *et al.,* 2005, Sayin *et al.,* 2009 and Zia-ur-Rehman *et al.,* 2010). Susceptible animals usually die within 3-4 weeks as a result of wide spread lymphocytolysis (Ahmed *et al.,* 2011). It is mainly transmitted transstadial various members of tick vectors of family Ixodidae and cause heavy economic losses to commercial host animals in epidemic areas (Li *et al.,* 2014).

Theileriosis in sheep and goats is often chronic and produces a sub-clinical form of disease and occasionally develop into clinical disease especially during stress conditions. Among all the *Theileria* spp. *Theileria lestoquardi*, *T. uilenbergi* and *T. luwenshuni* cause malignant theileriosis in sheep and goats. The other species, *T. ovis* and *T. separata*, cause subclinical infection in small ruminants (Elmam and Taha 2015). Generally, the diagnosis of small ruminant

piroplasmosis is based on microscopical examination of blood smears, lymph node smears and clinical symptoms.

These methods are reliable in acute cases, but have limited value for chronic or subclinical cases, where only low numbers of piroplasms exists. An alternative method which is more sensitive and specific is PCR based diagnostic techniques. PCR-based detection of *Theileria*-specific genes is able to determine *Theileria* infections with high sensitivity and specificity (Altay *et al.,* 2005, Bami *et al.,* 2009 and Oura *et al.,* 2004). It aids direct detection of piroplasms in carrier animals and is also specific in identifying the organism involved.

Materials and Methods

A total of 780 whole blood samples and peripheral blood smears were collected from sheep (360) and goats (420) from December 2016 to November 2017 in different seasons of Tamil Nadu. DNA was isolated from blood using DNA extraction kit (Qiagen, Germany). 18Sribosomal RNA gene of *Theileria* spp. was targeted for its detection. A primer set of forward:5'AGTTTCTGACCTATCAG 3'/ reverse:5' TTGCCTTAAACTTCCTTG 3' was used for amplification (Allsopp *et al.,* 1993). PCR was carried out in Bio-Rad T100™ Thermal Cycler.

Each 20µl reaction mixture comprised of 5µl template DNA, 10µl Master Mix (Red Ampliqon, US), 1µl of each primer (Forward and Reverse) and 3µl nuclease free water.

The PCR cycling conditions includes initial denaturation 94°C for 3 min; followed by 30 cycles of denaturation 94°C for 30 s, annealing 56°C for 1 min and extension 72°C for 1 min; with a final extension of 72°C for 5 min. The amplified PCR products were visualized in 1.5% agarosegel using gel documentation system (Bio Rad., USA).

Results and Discussion

The DNA extracted from the blood samples was amplified with the genus specific primers and later by species specific primers for *Theileria*. The amplicon of genus specific *Theileria* spp. was observed as a single band of 1098 bp(Fig 1). The results showed that 75.76 per cent (591/780) blood samples were positive for *Theileria* spp. by PCR. *Theileria* spp. infection was observed in 76.94 per cent (277/360) of sheep and 74.76 per cent (314/420) of goats in blood(Table 1). Statistical analysis of the observations from the present study was found to be higher than studies conducted by Aktas *et al.,* 2005, Durrani *et al.,* 2011, Riaz and Tasawar 2017 and Ullaha *et al.,* 2018who reported 54 per cent, 41 per cent, 35 per cent, 36 per cent and 14 per cent respectively whereas higher prevalence than the present study was recorded by Jalali *et al.,* 2013 and Mamatha *et al.,* 2017 who reported 89 per cent and 96 per cent respectively.

In the seasonal prevalence of *Theileria* spp. by PCR in sheep revealed that high prevalence was found during Summer season (90.00%) followed by South West monsoon (81.11%), North East monsoon (76.66%) and Winter season (60.00%) and in goats also high prevalence was noticed during Summer season (87.61%) followed by South West monsoon (76.19%), North East monsoon (71.42%) and Winter season (63.80%).

Among the seasons the present study showed that the theileriosis in sheep and goats was maximum in summer (90 and 87 per cent respectively) followed by monsoon and minimum in winter.

Table.1 Prevalence of *Theileria* spp. in sheep and goats by PCR

Species	No of animals examined	No of animals positive by PCR	Prevalence (%)	χ^2
Sheep	360	277	76.94	**0.50**^{NS}
Goat	420	314	74.76	
Total	780	591	75.76	

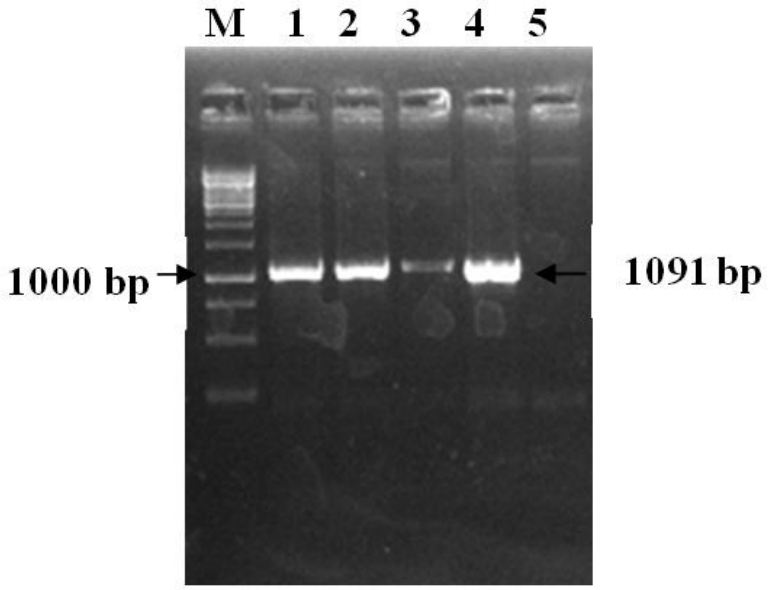
^{NS} Non-significant (p>0.05), * Significant (p<0.05), ** Highly significant (p<0.01)

Table.2 Seasonal prevalence of *Theileria* spp. by PCR in sheep and goat

Species/ Season	Winter season	Summer season	Southwest monsoon	Northeast monsoon	χ^2
Sheep	60.00% (54/90)	90.00% (81/90)	81.11% (73/90)	76.66% (69/90)	**24.10****
Goat	63.80% (67/105)	87.61% (92/105)	76.19% (80/105)	71.42% (75/105)	**16.61****

^{NS} Non-significant (p>0.05), * Significant (p<0.05), ** Highly significant (p<0.01)
Parenthesis indicates positivity out of total number of animals

Fig.1 PCR for the identification of *Theileria* spp. in blood DNA from sheep and goats



Lane M – 1kb ladder (Thermo scientific)
Lane 1 to 3 – Blood DNA samples
Lane 4 – Positive control
Lane 5 – Negative control

This result was in agreement with Khan *et al.*, 2017 but contrary with the results of Ahmed *et al.*, 2003 and Hegab *et al.*, 2016.The high prevalence during the summer months is attributed to hot and humid climate during summer season as tick infestations was

reported to be influenced by rainfall, temperature and humidity (Ghosh *et al.*, 2007). Moreover, during these months animals travel long distances for grazing and mixed with other flocks in grazing fields hence, share transmission of infections. The lower rates during winter months were due to the reason that animals were kept in captivity and there were less population of ticks. These results were in line with Irshad *et al.*, 2010 and Soundararajan *et al.*, 2014, 2018 who reported the highest prevalence of ticks during the summer season. The season wise prevalence revealed that, the highest prevalence was observed in summer months followed by South West monsoon, North East monsoon and winter season. It helps to diagnose and understand the epidemiology of theileriosis in small ruminants.

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