The Pharma Innovation Journal 2023; 12(3): 1954-1958

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(3): 1954-1958

www.thepharmajournal.com
Received: 02-01-2023
Accepted: 04-02-2023

Edith R
Department of Veterinary Parasitology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India

Harikrishnan TJ
Department of Veterinary Parasitology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India

Balagangatharathilagar M
Department of Veterinary Clinical Medicine
Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India

Corresponding Author:
Edith R
Department of Veterinary Parasitology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India

Molecular identification of *Theileria orientalis* genotypes and analysis of its phylogenetic relationship

Edith R, Harikrishnan TJ and Balagangatharathilagar M

Abstract
Oriental theileriosis is a non-lymphoproliferative, tick borne haemoprotozoan disease caused by *Theileria orientalis*. Subclinical cases of *T. orientalis* occurs commonly but stress may result in clinical cases of Oriental theileriosis. Many genotypes of *T. orientalis* have been identified and few of them have been associated with clinical disease. In this study, three major genotypes *viz.*, Chitose, Ikeda and Buffeli of *T. orientalis* were identified and sequenced based on Major Piroplasm Surface Protein (MPSP) gene. The sequences of these three genotypes were analyzed for its phylogenetic relationship. Evolutionary analysis of sequences of Chitose, Ikeda, Buffeli and 48 references from NCBI Gene Bank Database was done using MEGA6 tool. Phylogenetic trees of *T. orientalis* genotypes were generated by a stochastic algorithm. Initial tree(s) for the heuristic search were obtained by the Neighbour-Joining method to a matrix of pairwise distances estimated using the maximum composite likelihood approach. Phylogenetic analysis revealed that all these three genotypes of *T. orientalis* were closely related to *T. orientalis* of Brazil (AB581599), Vietnam (AB560823), Mangolia (AB571887) and Indonesia (AF 102500). Movement of animals from these places to India during ancient days might be the reason for this phylogenetic relationship of *T. orientalis* genotypes from the study area.

Keywords: *Theileria orientalis*, phylogenetic relationship, MPSP gene, Chitose, ikeda, buffeli genotypes

Introduction
Theileria orientalis a non-lymphoproliferative tick borne hemoprotozoan parasite of cattle and buffaloes has gone from the status of an incidental finding to a disease of its own, the Oriental theileriosis. Oriental theileriosis is characterized by haemolytic anaemia, jaundice, late term abortion in pregnant animals and death [1].

The disease has been reported to occur as outbreaks in many countries *viz.*, New Zealand, Australia and Japan [1, 2]. In the recent past, there was a severe outbreak of oriental theileriosis in India [3, 4]. Although subclinical cases of *T. orientalis* infection is common, stress may accelerate multiplication of the parasites resulting in clinical diseases [5, 6].

Many genotypes of *T. orientalis* have been identified *viz.*, Types 1-8 and N1-N3 and genotypes are defined based on the sequences of MPSP- an immunodominant antigen. Of many MPSP genotypes Chitose (Type-1) and Ikeda (Type-2) have been associated with clinical disease. The Buffeli (Type-3) is considered as benign while the clinical relevance of remaining types have not been clearly elucidated [7].

Recent research findings have shown that severity of the disease is dependent on genotypes which vary in their pathogenicity. Therefore, genotype identification of *T. orientalis* has been considered to be essential in every geographical location.

T. orientalis has been reported from Kerala, Odhissa and Uttar Pradesh but no data on genotype of *T. orientalis* in Tamil Nadu is available at present. Hence, the present study was conducted to identify *Theileria orientalis* genotypes using MPSP gene specific primers and to establish the phylogenetic position of *Theileria orientalis* genotypes.

Materials and Methods
Collection of blood samples: Blood samples were collected from 483 cross bred cattle from different regions of Tamil Nadu by jugular venipuncture in ethylene diamine tetra acetate (EDTA). The samples were stored in freezer under -20 °C deep freezer until the extraction of DNA. The blood smears were made from ear peripheral capillary blood. The blood smears stained with Giemsa stain and examined under microscope for *Theileria* sp.

Extraction of DNA: The DNA from blood samples was extracted using Blood Tissue DNA extraction (Qiagen) kit as per the standard procedure. The DNA extracted was stored in -20 °C.

Nested PCR using 18S rRNA

DNA samples were screened for *Theileria orientalis* infection using primers targeting the 18srRNA gene by Nested PCR. Samples that were positive by Nested PCR were utilized for *T.orientalis* genotype identification. Primers were designed for three major genotypes - Chitose, Ikeda and Buffeli of *T. orientalis* using MPSP gene [8, 9]. The primers designed for the three genotypes were

UF 5'GCCAACGACGTCGTTTTTACTGCC 3'
BF 5'TGATGAGAGATTCAAGGA 3'
IR 5' GCCAAGCACTGTTCATG 3'
CR 5' AGAACAGCGCTGAGGA 3'
BR 5' GAGATAGAAGAATACTGCAAAGGAG 3'

The thermal cycling programme was done at an initial denaturation-95 °C for 3 minutes, followed by 30 cycles of denaturation at 95 °C for 40 s; annealing at 55 °C for 30s; extension at 72 °C for 1 min; a final extension at 72 °C for 5 min and then hold at 4 °C. Amplicons of different genotypes were excised from ethidium bromide stained gels and purified using the Qiagen Gel Extraction Kit and sent for sequencing to Eurofins Genomics India Pvt. Ltd, Bangaluru, Karnataka, India.

Phylogenetic Analysis

Evolutionary analysis of three major genotypes of *T. orientalis* was conducted in MEGA6 tool. Phylogenetic analysis included a total of 51 nucleotide sequences. In this study, three sequences of chitose, ikeda and buffeli and 48 reference sequences from the NCBI GenBank database have been used. Phylogenetic trees of *T. orientalis* subtypes were generated by a stochastic algorithm, which uses maximum likelihood (ML) to simultaneously search for the best tree topology, branch lengths, and nucleotide substitution model parameters. Initial tree (s) for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach [8, 9].

Results

Out of 483 samples screened by blood smear examination, five samples were positive for *T. orientalis* whereas, 61 samples were positive for *T. orientalis* by PCR. The overall prevalence of *T. orientalis* recorded in this study was 12.63 percent with the maximum prevalence of *T. orientalis* in Chennai district and minimum in Kanyakumari district of Tamil Nadu.

Table 1: Prevalence of *T. orientalis* in Tamil Nadu, India

Location	No. of samples screened	No. positive	Prevalence (percent)
Chennai	227	49	21.59
Madurai	64	6	9.37
Kanchipuram	62	2	3.22
Thiruvallur	20	2	10.00
Tenkasi	70	2	2.86
Kanyakumari	40	0	0
Total	483	61	12.63

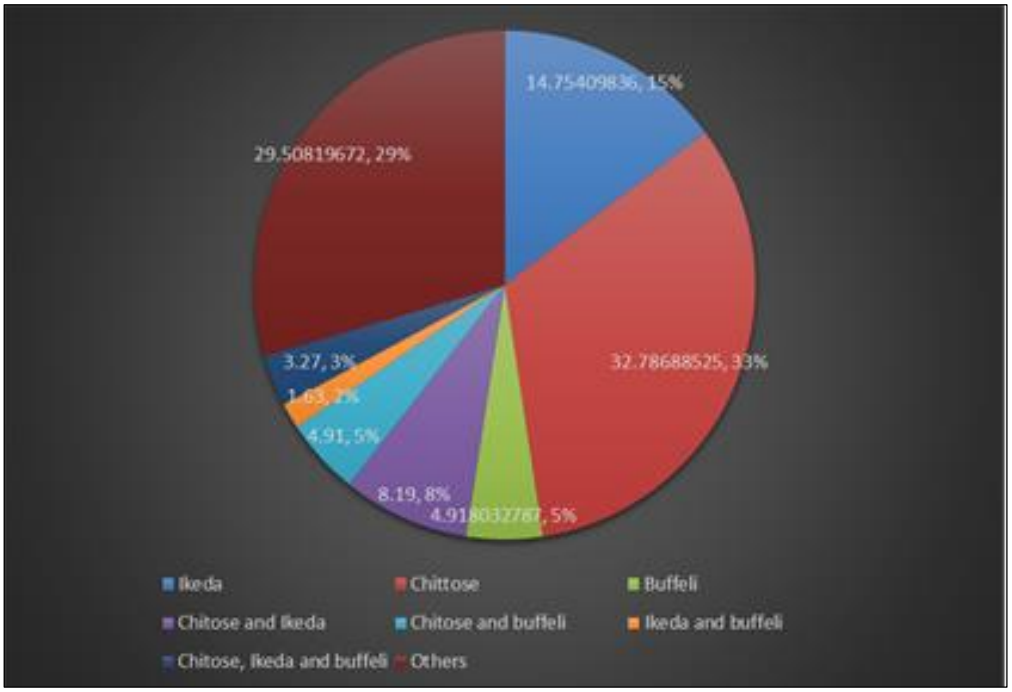

Fig 1: Prevalence of genotypes of *Theileria orientalis*

Subtype Identification

Three major genotypes were screened by PCR. Out of 61 *T. orientalis* samples, 9, 20 and 3 were positive for only Ikeda, Chitose and Buffeli respectively. Five samples were positive for Chitose and Ikeda, 3 positive for Chitose and Buffeli and one sample was positive for Ikeda and Buffeli. Two samples out of 61 were positive for three genotypes whereas18 samples have shown no amplification for any of these three genotypes.

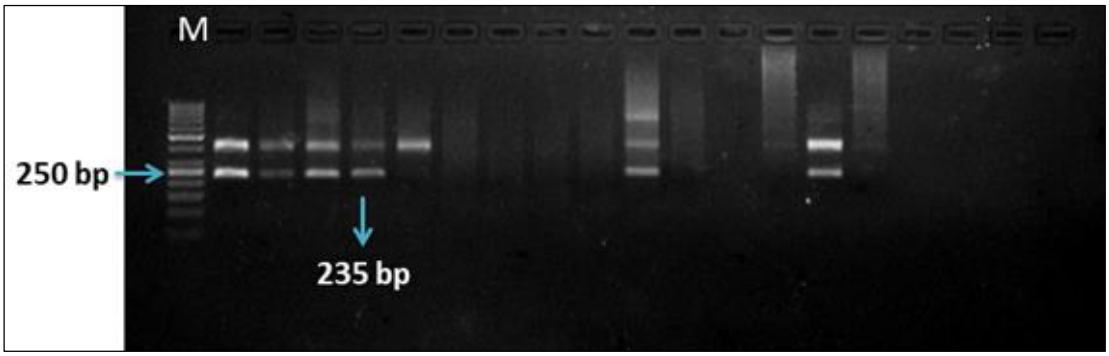

Fig 2: *Theileria orientalis* (235 bp)

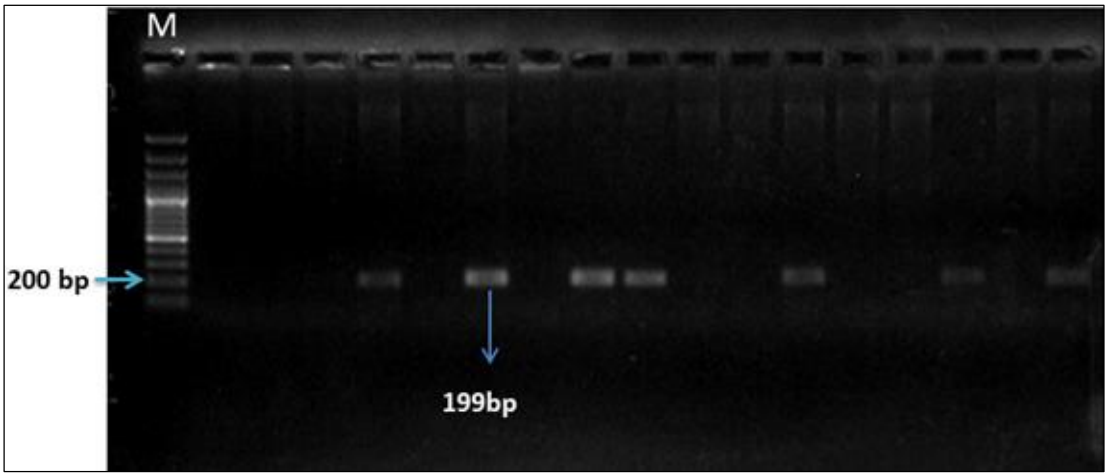

Fig 3: *Theileria orientalis* (Buffeli-199 bp)

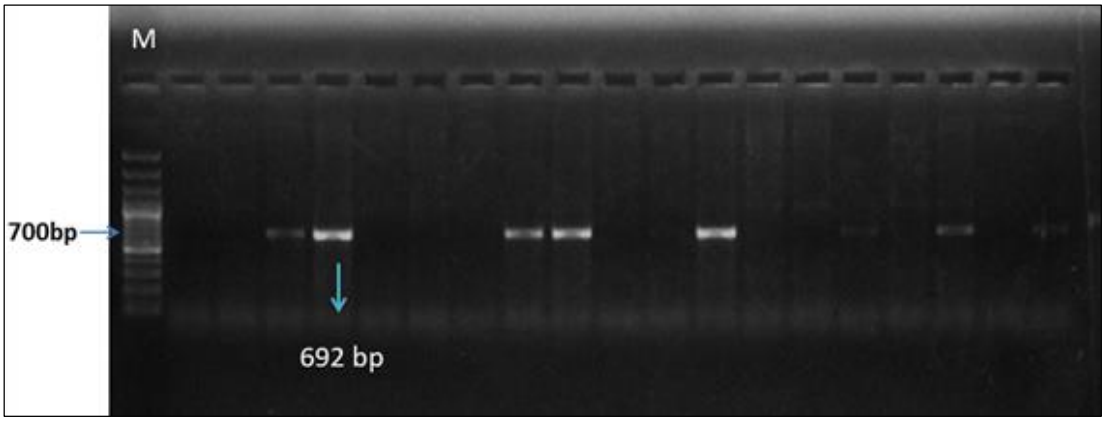

Fig 4: *Theileria orientalis* (Chitose 692bp)

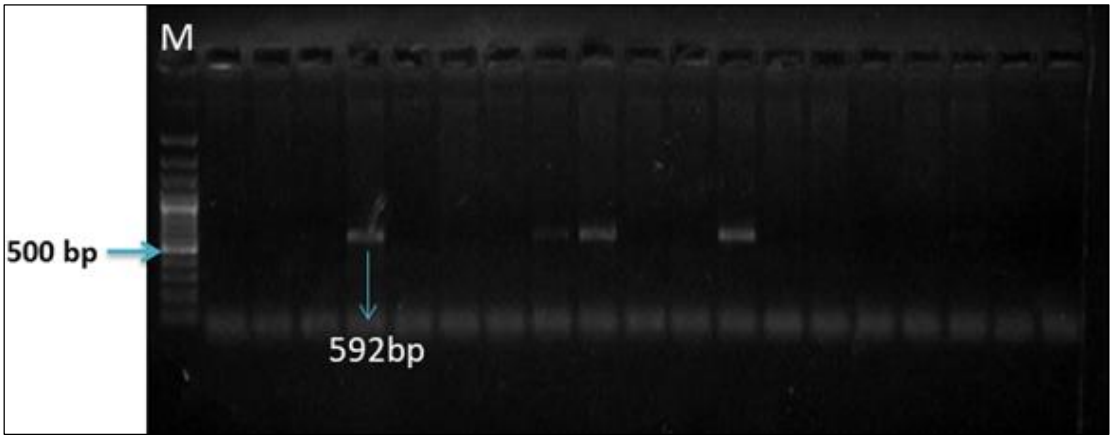

Fig 5: *Theileria orientalis* (Ikeda 592bp)

Phylogenetic Analysis

Initial tree(s) for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.8910)). All these three Chennai isolates of chitose, ikeda and buffeli were closely related to *Theileria orinetalis* of Brazil (AB581599), Viatnam (AB560823), Mongolia (AB571887) and Indonesia (AF102500).

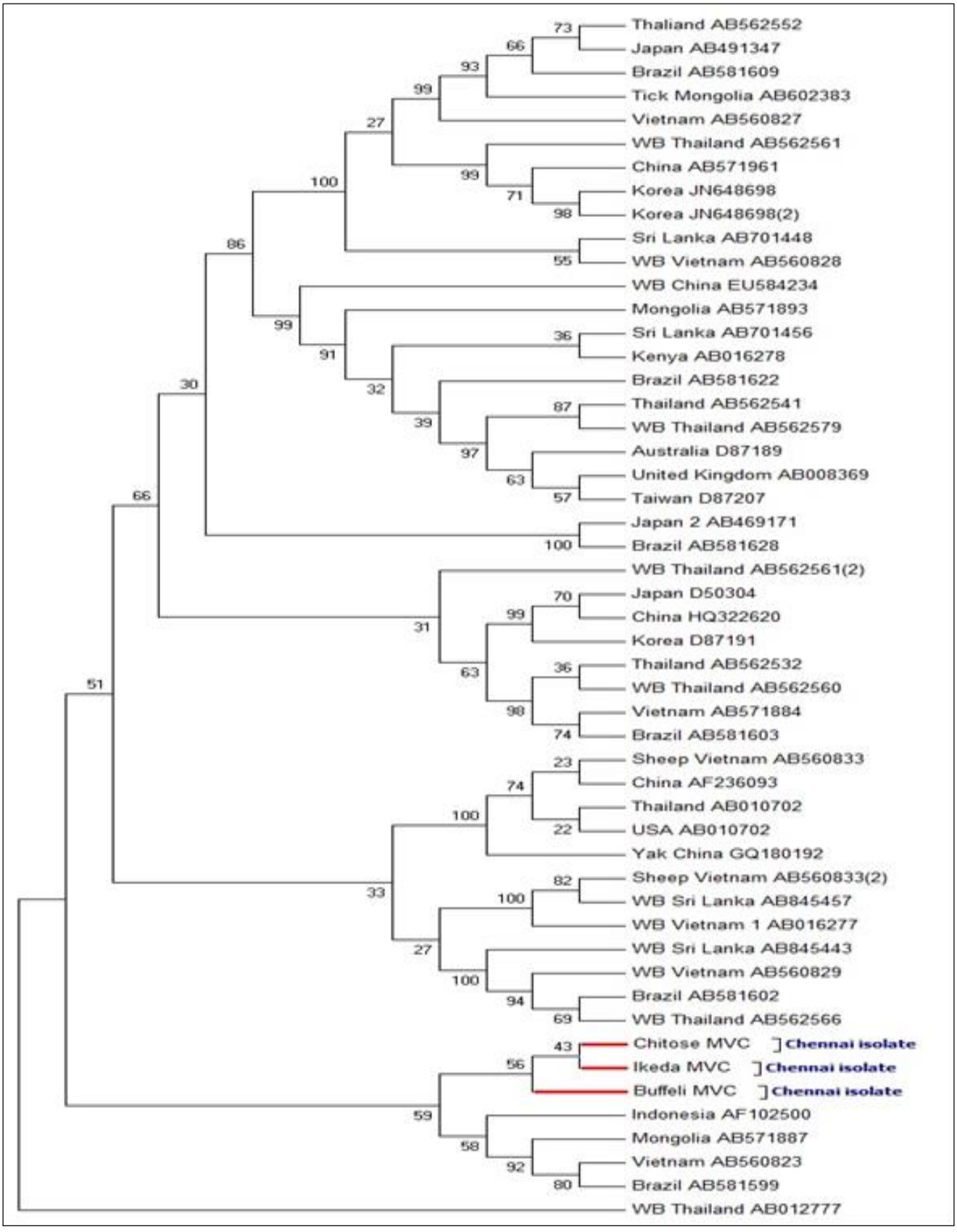

Fig 6: Phylogenetic analysis

Discussion

T. orientalis has been incriminated for causing benign infection, fever, anemia, abortion in pregnant cows, lethargy jaundice and occasionally high fatality in many countries including India in the recent past. It has been reported that both clinical and subclinical Oriental theileriosis occur as a mixture of genotypes [1, 7, 8]. The present study explored the prevalence of *T. orientalis* and identified the genotypes of *T. orientalis* based on MPSP gene in Tamil Nadu. Three major genotypes of *T. orientalis* viz. Ikeda, Chitose and Buffeli were identified from the study area as a single or mixed infection. In this study also mixture of three major genotypes of *T. orientalis viz.*, Chitose, Ikeda and Buffeli were observed.

Out of these three genotypes, Chitose had the highest prevalence followed by Ikeda and Buffeli the least It was not clear whether these mixed infections arose from successive inoculations of distinct parasite populations form multiple tick bites or transmission of multiple sporozoite genotypes from individual ticks. Studies suggest that genetic recombination within the vector is sufficient to generate a genetically diverse population of sporozoites [8]. Multiple MPSP genotypes have been detected within *T. orientalis* sporozoites harvested from *H. longicornis* ticks suggesting that mixed genotypes of this can be transmitted in a single infective bite resulting in temporal switching of genotypes [10].

Many studies have clearly linked the Ikeda genotype of *T. orientalis* to clinical disease whereas the pathogenicity of the Chitose genotype is less clear. Restricted immune response similar to *T. parva* and *T. annulata* infection could explain the relative pathogenicity of different genotypes. The differential host response to *T. orientalis* could be another factor [2].

Although clinical Oriental Theileriosis has not been observed in this study, stress such as transport, pregnancy, lactation etc., can act as a trigger for clinical outbreak of disease. Eighteen samples found positive for *T. orientalis* did not amplify and found negative for three major genotypes studied in this area presumably genotype (s) other than the three identified.

Phylogenetic analysis had shown that the *T. orientalis* Chennai isolates were closely related to Brazil, Vietnam, Mongolia and Indonesia. Movement of animals from these places to India during ancient days could explain this phylogenetic relationship of *T. orientalis* isolates from the study area.

Conclusion

It is concluded that three major genotypes of *T. orientalis* are documented in Tamil Nadu *viz.*, Chitose, Ikeda and Buffeli. Ikeda, the pathogenic genotype is less prevalent than the other two major genotypes. Subclinical / carrier status of the disease has been observed in this study. Further study is warranted to find out the prevalence of genotypes other than the three major genotypes of *T. orientalis* documented in this study.

References

1. Bogema DR, Deutscher AT, Fell S, Collins D, Eamens GJ, Jenkins C. Development and validation of a multiplxed hydrolysis probe qPCR assay for the detection and quantification of *Theileria orientalis* and differentiation of clinically relevant subtypes. J Clin. Microbiol. 2015;53:941-950.
2. Yang Y, Mao Y, Kelly P, Yang Z, Luan L, Zhang J, *et al.* A pan-Theileria FRET-qPCR survey for Theileria spp. in ruminants from nine provinces of China. Parasit. Vectors. 2014;7:413.
3. Aparna M, Reghu Ravindran MB, Vimalkumar, Bindu Lakshmanan P, Rameshkumar KG, Ajith Kumar, K. Promod, *et al.* Molecular characterization of *Theileria orientalis* causing fatal infection in crossbred adult bovines of South India. Parasitology International. 2011;60(4):524-529.
4. Selim, Ahmed Magdy, Santosh Kumar Senapati, Manoranjan Das, Chinmoy Mishra, Ramesh Chandra Patra, Susen Kumar Panda. Molecular, epidemiological and haematological evaluation in *Theileria orientalis* infected cattle from an endemic region in India. Animal Biotechnology. 2021;32(6):663-670.
5. George, Neena, Vasundhra Bhandari, D Peddi Reddy, Paresh Sharma. Emergence of new genotype and diversity of *Theileria orientalis* parasites from bovines in India. Infection, Genetics and Evolution. 2015;36:27-34.
6. Vinodkumar, Kulangara, Varikkottil Shyma, Davis Kollannur Justin, Sivasailam Ashok, *et al.* Fatal *Theileria orientalis* N2 genotype infection among Asian water buffaloes (*Bubalus bubalis*) in a commercial dairy farm in Kerala, India. Parasitology. 2016;143:69-74.
7. Eamens GJ, Bailey G, Gonsalves JR, Jenkins C. Distribution and temporal prevalence of *Theileria orientalis* major piroplasm surface protein types in Eastern Australian cattle herds. Aust. Vet. J. 2013;91:332-340.
8. Nkhoma SC, Nair S, Cheeseman IH, Rohr-Allegrini C, Singlam S, Nosten F, Anderson TJC. Close kinship within multiple-genotype malaria parasite infection. Proc. R. Soc. B. 2012;279:2589-2598.
9. Sivakumar T, Hayashida K, Sugimoto C, Yokoyama N. Evolution and genetic diversity of *Theileria*. Infec. Genet. Evol. 2014;27:250-263.
10. Kubota S, Sugimoto C, Onuma M. Population dynamics of *Theileria sergenti* in persistently infected cattle and vector ticks analysed by a polymerase chain reaction. Parasitology. 1996;112:437-442.