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Molecular Prevalence of *Theileria annulata* in Cattle from Different Agroclimatic Zones of Tamil Nadu, India

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A B S T R A C T

Bovine tropical theileriosis caused by *Theileria annulata* is an economically important disease of cattle in tropical and subtropical countries. Conventional diagnostic methods are unable to identify the subclinical and carrier status of this disease. Molecular diagnostic methods are more sensitive in detection of subclinical and carrier condition on the disease. In this study 817 blood samples were collected from cattle of seven different agroclimatic zone of Tamil Nadu state. DNA extracted from these blood samples were screened by nested Polymerase Chain reaction using primers targeting the partial region of 18SrRNA gene of *T. annulata*. 114 samples were positive out of 817 samples. North eastern zone showed more prevalence (20.57 %) followed by North western zone (18.80%) whereas hilly zone (3.23%) showed least prevalence of *T. annulata* in cattle. Present study shows that the 18S rRNA based molecular screening of *T. annulata* infection in cattle is useful to assess the prevalence of bovine tropical theileriosis.

Introduction

Theileria annulata is an apicomplexan tick borne protozoan which causes Tropical Bovine Theileriosis. In tropical countries, it is an economically important disease and one of the major obstacles for the improved livestock production particularly in exotic and cross bred dairy cattle in tropical and subtropical countries including India (Minjauw and McLeod, 2003).

The losses are not only due to clinical disease and mortality but also due to carrier status of the disease associated production losses in terms of delay in growth, reproduction and milk production (Gharbi *et al.*, 2011). The adverse effects of the disease are more

prominent in crossbred cattle compared to the indigenous cattle population.

In India, several reports documented bovine tropical theileriosis from subclinical to severe clinical outbreaks (Shastri *et al.,* 1980; Bansal *et al.,* 1987). The prevalence of this disease in cross bred cattle was reported from Karnataka (Ananda *et al.,* 2009), Kerala (Nair *et al.,* 2011), odhisa (Acharya *et al.,* 2017), Punjab (Shahnawaz *et al.,* 2011), Tamil Nadu (Velusamy *et al.,* 2014) and Uttrakhand (Kohli *et al.,* 2014) based upon blood smear studies and serological studies.

The conventional diagnosis of theileriosis is based on history of tick infestation, clinical signs and examination of Giemsa stained blood and lymphnode aspiration smears (Mans *et al.,* 2015). These methods become unreliable when there is subclinical and / or carrier status of the disease. The advent of molecular techniques like polymerase chain reaction has revolutionized the scenario from unreliable to more sensitive and specific detection of infections including the carrier status.

In this study, a qualitative PCR has been used to study the molecular epidemiology of tropical bovine theileriosis in the different agroclimatic zones of Tamil Nadu.

Materials and Methods

Study region and animal population

The present study was carried out in seven agro climatic regions of Tamil Nadu state of India (Fig. 1 and Table 1). Tamil Nadu is the Southernmost state of India. It is located between 8.05' and 13.34' North latitudes and 76.14' and 80.21 East longitudes. It covers an area of about 13 Mha. and accounts for about 4 per cent of the total geographical area of the country. The Tamil Nadu State forms part of the peninsular shield and composed of geologically ancient rock of diverse origins (i.e different soils). About three fourth of the area of the state is unclassified crystalline rocks of Archaeon age and the rest is sedimentary rocks.

The State can broadly be divided into seven agro-climatic zones. The climate is semi-arid in the plains and humid to sub-humid in the hills with annual rainfall from 750 mm in some parts of the plains to over 2400 mm in the high hills.

The study was carried out in dairy cattle between April 2015 and September 2018. Blood samples were collected in EDTA anticoagulant tubes by jugular venipuncture from cattle selected randomly. The cattle selected for *Theleria annulata* testing were aged between 6 months and 12 years. The blood samples were stored at -20^{o}C until further use.

DNA extraction

The DNA was extracted from whole blood using a Qiagen Blood DNA Kit. Briefly, 200 μL of blood was mixed with 20 μL proteinase K with this 200 μL of lysis buffer (AL) was added and mixed thoroughly by vortexing and incubated at 56^{o}C for 10 minutes. 200μL of ethanol (100%) was added. This mixture was transferred to the DNeasy Mini Spin Column placed in a 2ml collection tube and centrifuged at 8000rpm for 1 minute. The flow through were discarded and the spin column was placed in new 2ml collection tube. 500μL of wash buffer 1 (AW1) was added to the column and again centrifuged at 8000 rpm for 1 minute. The spin column was again placed in a new 2ml collection tube and 500μL wash buffer 2 (AW2) was added to the column and centrifuged at 13,000 rpm for 3 minutes. After these two washings, the spin column was transferred to a new 1.5 ml micro

centrifuge tube. 30 μL of elution buffer (AE) was added to the spin column and was incubated for one minute at room temperature. The tubes were centrifuged at 8000 rpm for one minute. The flows through portion containing DNA were stored at -20^{o}C until further use.

Theileria annulata DNA amplification

Primary PCR was performed with a set of gene specific primers to amplify 416bppartial region of 18S rRNA gene of Theileria genus. Using this primary PCR product as template a nested PCR was performed with a species specific primers to amplify 193bp partial region of 18S rRNA gene of *Theileria annulata.* The PCR was carried out in 20 μL volume for each reaction consisting of 10 μL Red Eye Master Mix, 1 μL of forward primer and 1 μL of reverse primer, 1 μL template and 7 μL nuclease free water. The reactions were performed in a thermal cycler. For primary PCR the cyclical conditions were initial denaturation at 94^{o}C for 5 Minutes followed by 30 cycles of denaturation, annealing and extension (94^{o}C for45 sec., 67^{o}C for 1 min. 72^{o}C for 1 min) and a final extension at 72^{o}C for 5 min. The cyclical condition for nested PCR were initial denaturation at 94^{o}C for 5 min. followed by 30 cycles (94^{o}C for 45 Sec, 62^{o}C for 1 Min. and 72^{o}C for 1 min.) and a finalextension at 72^{o}C for 5 Min. Electrophoresis was performed in 1% agarose gel with ethidium bromides and visualized under Geldoc$^{®}$system (Fig. 2).

Statistical analysis

The observed prevalence was estimated as follows:

Prevalence (%) = {(number of infected animals)/(total number of examined animals)} X 100

Results and Discussion

Out of 817 blood sample screened for *Theileria annulata* infection, 114 samples were positive by PCR (13.95%, Table 1). Among the seven agroclimatic zones of Tamil Nadu Northeastern zone had shown highest prevalence (20.57 %) followed by North western zone (18.80%) whereas the high hilly fall zone had shown least prevalence (3.23 %) (Fig. 3). The high prevalence in the north eastern zone might be due to abundance of tick vectors and poor housing conditions.

Variation in the molecular prevalence of infection in cattle from different agroclimatic zones is directly related to the factors like vector prevalence, temperature and humidity.

The prevalence was higher in cattle aged >6 years (64/114) than cattle aged <3 years (19/114) and 3- 6 years (31/114). *T. annulata* infection of cattle was shown to be positively associated with age (Weir *et al.,* 2011). The higher incidence of *T. annulata* in cattle aged > 6 years might be due to increased attractiveness for ticks, multiple infections, hormonal changes, and high production stress due to calvings (Sutherst *et al.,* 1983; Kabir *et al.,* 2011).

Polymerase chain reaction assays are more specific and sensitive than microscopy and serological methods to study the epidemiological status of any protozoan infection.18S rRNA gene based PCR assays have been successfully applied by many researchers for quick and accurate diagnosis of *T. annulata* infection (Khan *et al.,* 2013; George *et al.,* 2015). 18S rRNA based quantitative PCR assay also has been done to quantify *T. annulata* infection (Chaisi *et al.,* 2013). Though diagnosis of Bovine Tropical Theileriosis can be done with conventional microscope based methods, it is unreliable when the parasite load in blood is less.

Fig.1 Agroclimatic zones of Tamil Nadu

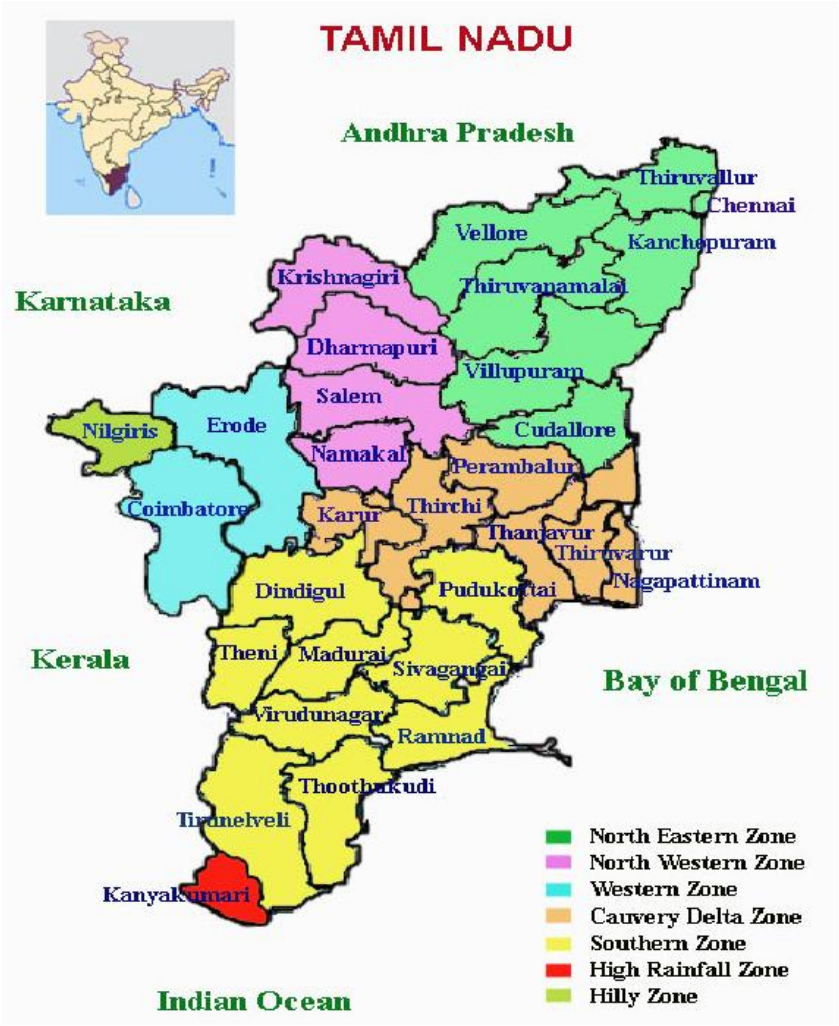

Fig.2 *Theileria annulata* nested PCR gel showing 193bp

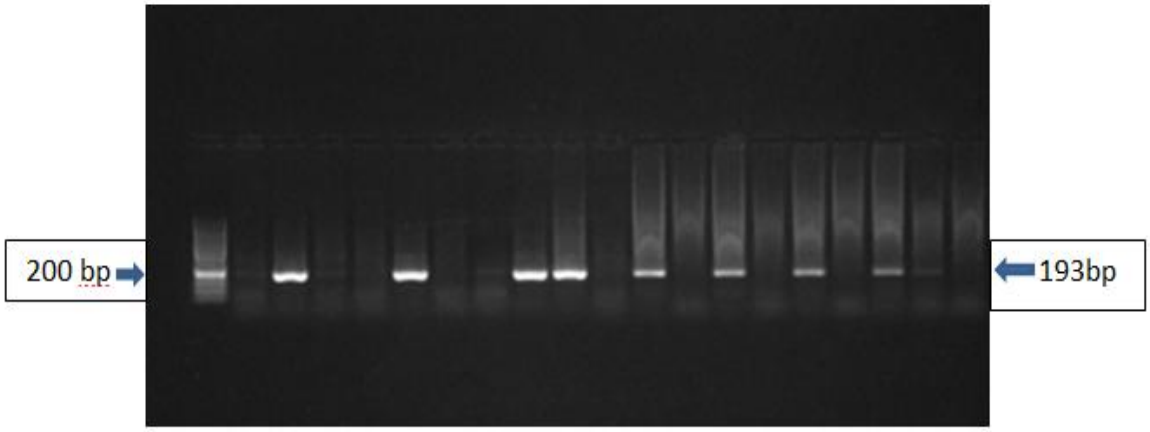

Fig.3 Molecular prevalence of *T. annulata* infection in Tamil Nadu

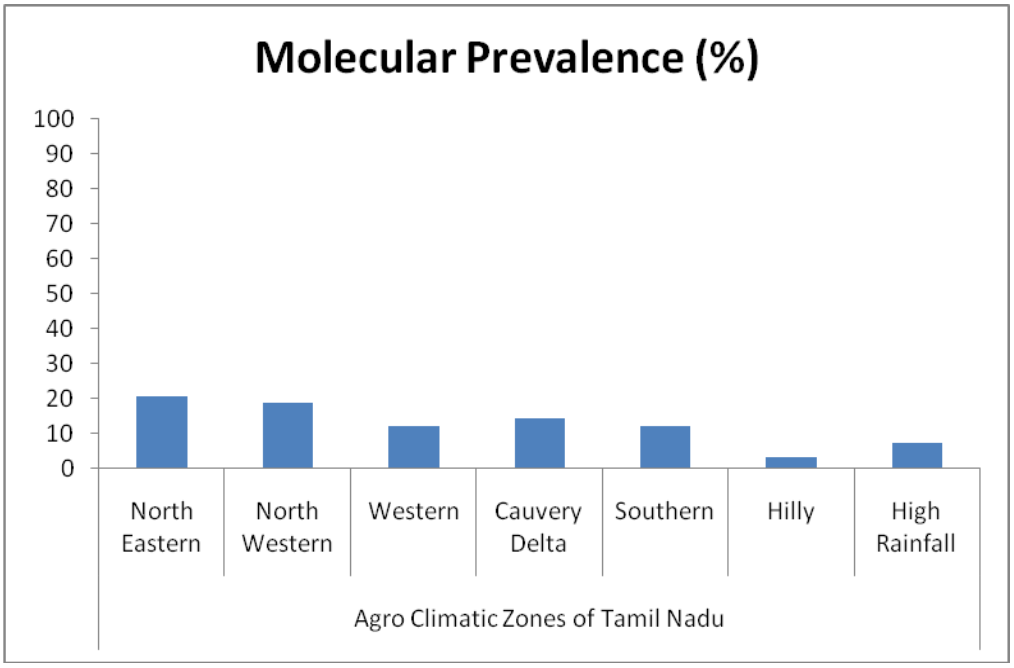

Table.1 Molecular prevalence of *Theileria annulata* in cattle from various agroclimatic zones of Tamil Nadu

Agroclimatic Zones	Places	Total Number of samples screened	Number of Samples Positive for *T. annulata* by PCR	Percent prevalence
North Eastern Zone	Chennai, Tiruvallur, Kancheepuram, Thiruvannamalai, Cauddalore	209	43	20.57
North Western Zone	Salem, Namakkal, Dharmapuri, Krishnagiri	117	22	18.80
Western Zone	Erode, Coimbatore	97	12	12.37
Cauvery Delta Zone	Karur, Trichy, Thiruvarur, Ariyalur	103	15	14.56
Southern Zone	Madurai, Tirunelveli	91	11	12.09
Hilly Zone	The Nilgiris, Kodaikkanal, Pannaikadu, Vathalagundu	93	3	3.23
High Rainfall Zone	Nagerkoil	107	8	7.47
	Total	817	114	13.95

The carrier status of infection is the major source of spreading the infection to healthy population. Prevalence studies based on molecular methods will help in accurate diagnosis of any state of infection is need of the hour for planning better control and prevention of the disease

The result of the present study has proved that PCR amplification of 18S rRNA gene of *T. annulata* from blood of bovine is a useful tool to assess the molecular epidemiological status of the bovine tropical theileriosis.

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References

Acharya, A.P., Panda, S.K. and Prusty, B.K., 2017. Diagnosis and confirmation of *Theileria annulata* infection in cattle in Odisha, India. *Journal of Entomology and Zoology Studies*, *5*(4), pp.1543-1546.

Ananda, K.J., D'Souza, P.E. and Puttalakshmamma, G.C., 2009. Prevalence of haemoprotozoan diseases in crossbred cattle in Banglore north. *Veterinary World*, *2*(1), p.15.

Bansal, G.C., Ray, D., Shrivastav, R.V.N., Subrahmanian, G., 1987. Sero-prevalence of bovine theileriosis in some farms in India. Indian J. Anim. Sci. 57: 366–368

Chaisi, M. E., Janssens, M. E., Vermeiren, L., Oosthuizen, M. C., Collins, N. E., & Geysen, D. (2013). Evaluation of a real-time PCR test for the detection and discrimination of Theileria species in the African buffalo (*Synceruscaffer*). *PloS one*, *8*(10), e75827.

George, N., Bhandari, V., Reddy, D.P. and Sharma, P., 2015. Molecular and Phylogenetic analysis revealed new genotypes of *Theileria annulata* parasites from India. *Parasites & vectors*, *8*(1), p.468.

Gharbi, M., Touay, A., Khayeche, M., Laarif, J., Jedidi, M., Sassi, L. and Darghouth, M.A., 2011. Ranking control options for tropical theileriosis in at-risk dairy cattle in Tunisia, using benefit-cost analysis. *Revue Scientifique et Technique-OIE*, *30*(3), p.763.

Kabir, M.H.B., Mondal, M.M.H., Eliyas, M., Mannan, M.A., Hashem, M.A., Debnath, N.C., Miazi, O.F., Kashem, M.A., Islam, M.R. and Elahi, M.F., 2011. An epidemiological survey on investigation of tick infestation in cattle at Chittagong District, Bangladesh. *African Journal of Microbiology Research*, *5*(4), pp. 346-352.

Khan, M.K., He, L., Hussain, A., Azam, S., Zhang, W.J., Wang, L.X., Zhang, Q.L., Hu, M., Zhou, Y.Q. and Zhao, J., 2013. Molecular epidemiology of *Theileria annulata* and identification of 18S rRNA gene and ITS regions sequences variants in apparently healthy buffaloes and cattle in Pakistan. *Infection, Genetics and Evolution*, *13*, pp.124-132.

Kohli, S., Atheya, U.K., Srivastava, S.K., Banerjee, P.S. and Garg, R., 2014. Outbreak of theileriosis and anaplasmosis in herd of holstein crossbred cows of Dehradun district of Uttranchal, India: A Himalayan region. *International Journal of Livestock Production*, *5*(1), pp.6-9.

Mans, B.J., Pienaar, R. and Latif, A.A., 2015. A review of Theileria diagnostics and epidemiology. *International Journal for Parasitology: Parasites and Wildlife*, *4*(1), pp.104-118.

Minjauw, B. and McLeod, A., 2003. Tick-borne diseases and poverty: the impact of ticks and tick-borne diseases on the livelihoods of small-scale and marginal livestock owners in India and eastern and southern Africa. *Tick-borne diseases and poverty: the impact of ticks and tick-borne diseases on the livelihoods of small-scale and marginal livestock owners in India and eastern and southern Africa.*

Nair, A.S., Ravindran, R., Lakshmanan, B., Kumar, S.S., Tresamol, P.V., Saseendranath, M.R., Senthilvel, K., Rao, J.R., Tewari, A.K. and Ghosh, S., 2011. Haemoprotozoa of cattle in northern Kerala, India.*Tropical Biomedicine*, *28*(1), pp.68-75.

Shahnawaz, S., Ali, M., Aslam, M.A., Fatima, R., Chaudhry, Z.I., Hassan, M.U. and Iqbal, F., 2011. A study on the prevalence of a tick-transmitted pathogen, *Theileria annulata*, and hematological profile of cattle from Southern Punjab (Pakistan). *Parasitology research*, *109*(4), p.1155.

Shastri, U.V., Deshpande, P.D., Deshpande, M.S., 1980. Some observations on *Theileria mutans* like heamoprotozoa parasite of cattle of Maharastra. In: Gautam OP, Sharma RD, Dhar S (eds) Proceedings of the seminar on haemoprotozoan disease of domestic animals, Hisar, India. Haryana Agricultural University, Hisar, India, p 83.

Sutherst, R.W., Kerr, J.D., Maywald, G.F. and Stegeman, D.A., 1983. The effect of season and nutrition on the resistance of cattle to the tick *Boophilus microplus*. *Australian Journal of Agricultural Research*, *34*(3), pp.329-339.

Velusamy, R., Rani, N., Ponnudurai, G., Harikrishnan, T.J., Anna, T., Arunachalam, K., Senthilvel, K. and Anbarasi, P., 2014. Influence of season, age and breed on prevalence of haemoprotozoan diseases in cattle of Tamil Nadu, India.

Weir, W., Karagenç, T., Gharbi, M., Simuunza, M., Aypak, S., Aysul, N., Darghouth, M.A., Shiels, B. and Tait, A., 2011. Population diversity and multiplicity of infection in *Theileria annulata*. *International journal for parasitology*, *41*(2), pp.193-203.

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