

DETECTION OF *Theileria* AND *Anaplasma* spp. IN CARRIER BUFFALOES
(*Bubalus bubalis*) BY POLYMERASE CHAIN REACTION IN TAMIL NADU, INDIA

Gurusamy Ponnudurai*, Natarajan Rani, Balan Rubinibala,
Alagarsamy Alagesan and Ramasamy Saravanan

Received: 20 March 2020

Accepted: 30 November 2022

ABSTRACT

A total of 150 heparinised blood samples and equal number of blood smears were collected from buffaloes in three agro-climatic zones of Tamil Nadu viz., Cauvery delta, North - western and Southern zones to detect *Theileria* and *Anaplasma* by blood smear examination and Polymerase Chain Reaction. The blood smear examination revealed that none of animals were found to harbour any haemoprotozoan parasites. Whereas PCR detected *Theileria* and *Anaplasma* in 26.9% and 48.0%, and 10% and 15% of the animals, respectively in north- western zone and Cauvery delta, however no animal from south zone was detected positive for any protozoan. Besides, mixed infection of *Theileria* and *Anaplasma* was recorded in 8 (5.3%) animals. The analysis of data collected to determine the risk factors associated with occurrence of haemoprotozoan parasites revealed that the pure breed Murrah showed higher percentage of *Theileria* and *Anaplasma* positivity (30.7% and 46.1%) than non - descriptive breed (8.7% and 16.0%). The percentage of positivity of haemoprotozoan parasites was found to be higher among animals of 1 year, followed by 2 years and > 4 years of age.

Keywords: *Bubalus bubalis*, buffaloes, *Theileria*, *Anaplasma*, PCR, detection, India

INTRODUCTION

India has 56% of the world buffalo population, and it shares about 64% of total buffalo milk production of the world (FAO report, 2000). Since buffaloes have a unique ability to thrive on low quality crop residues and green fodder, the small and marginal farmers prefer to maintain them as a small herd in mixed farming system for manure and milk (Resali, 2000). In India, several breeds of buffaloes are being maintained, but only 15 breeds have been described, while remaining population referred to as non- descriptive breed. There has been a notion that buffaloes are resistant to many parasitic diseases; even if they are susceptible to infection, they do not show overt clinical manifestations especially in haemoprotozoan infection. Instead they serve as carrier and source of infection to tick vectors. In the ticks, the parasites multiply and become infective to susceptible crossbred cattle, causing economic losses to the farming community. It is difficult to detect carrier status

of haemoprotozoan parasites by conventional parasitological techniques as number of parasites will be low. The molecular tool PCR is shown to have high specificity and sensitivity in detecting carrier status; hence this study was undertaken to determine the carrier status of *Theileria* and *Anaplasma* in buffaloes in Tamil Nadu.

MATERIALS AND METHODS

Study area

The present study was undertaken from October'2014 to' August' 2015 in three agro climatic zones *viz.*, Cauvery delta zone (CDZ), North-western zone (NWZ) and Southern zone (SZ) located in the central part of Tamil Nadu, India. The climatic condition of the study areas is hot and humid (28.8°C) with annual rainfall of 764 mm and 860 mm in Cauvery delta and North-western zone respectively.

Sample and data collection

In this study, blood samples were collected from 150 apparently healthy animals, from randomly selected herd located in North-western Zone, Cauvery delta zone and Southern Zone. The blood was collected from the jugular vein of animals in the vacutainer coated with 0.5 M EDTA. Blood smears were also prepared from blood obtained from puncturing the ear vein. Data pertaining to breed, sex, age and presence / absence of tick infestations were collected through questionnaires to determine the risk factors associated with prevalence of theileriosis. In the tick infested animals, the ticks were counted and collected in a vial containing 70% ethanol.

Blood smear examination

Thin blood smears were fixed in methanol and stained with Giemsa stain (1 in 20 dilutions) for 40 minutes and examined under oil immersion. A minimum of 50 to 100 fields for each smear were examined for the presence of piroplasms of *Theileria* sp.

Molecular technique

DNA extraction

Genomic DNA was extracted from blood samples using a commercial DNA extraction kit (Qiagen, Chatsworth, CA, USA) according to the manufacturer's instruction and isolated DNA was stored at -80°C until further analysis.

PCR amplification

The nucleotide sequence of specific primers of *Theileria annulata* and *Anaplasma* species respectively ThaF-5' – ACGGAGTTCTTTGTCTGA-3' and ThaR -5'ACGGATTTCTTTGTCTGA-3' and AEUF – 5' – GGTTAATTCGATGCAACGCGA – 3' and ANA R- 5'- GCTCAGCCTTGCGACGTT-3' were designed and PCR amplification was performed in a final volume of 25 µl. Each reaction contained 4 µl of DNA template, 4.5 µl of Nuclease free water, 12.5 µl of 1.5 mM MgCl₂ (Taq 2X Master mix, Red Ampliqon) and 2 µl of each primer at 10 pmol. The DNA amplification was carried out in a thermal cycler (Eppendorf, Germany). Thermo-profile used by Junlong *et al.* (2015) was modified for this study and it consisted of 35 cycles of three steps each, comprising an initial denaturing at 95°C for 30 seconds, primer annealing at 55°C for 30 seconds and product extension at 72°C for 30 seconds. Amplified products were separated by electrophoresis on a 1% agarose gel and visualized under a Gel documentation system (BioRad, USA).

Statistical analysis

The data collected in this study were analysed by Chi square test to associate the risk factors such as zone, breed, and age with occurrence of *Theileria* and *Anaplasma* in buffaloes. Statistical analysis of data was done using SPSS package (Version 16) and $P < 0.05$ was considered as statically significant at 95% confidence interval.

RESULTS AND DISCUSSIONS

In this investigation, the blood smear examination did not reveal any positivity for *Theileria* and *Anaplasma*. Whereas PCR detected *Theileria* and *Anaplasma* in 26.9% and 48.0%, and 10% and 15% of animals, respectively in North-western and Cauvery delta zone, however no sample from Southern zone was detected positive for any protozoan. A predicted product size of 193 and 335 pb with *Theileria* and *Anaplasma* spp. respectively were detected (Figure 1 and Figure 2).

In the present study, *Theileria* spp. was detected in 26.9% and 10% of the samples from NWZ and CDZ respectively. The results are in partial agreement with findings of Kundave *et al.* (2015) who recorded *Theileria* spp. in 10.8% of the blood samples from buffaloes in Gujarat, India; however, the high prevalence of *Theileria* (26.9%) in NWZ in this study might be due to geographical influence on tick vectors. Despite conventional method of screening by blood smear examination, Malyar and Farid (2019) recorded *Theileria* spp. in 26.4% of buffaloes in Afghanistan, which is similar to the prevalence of infection observed in the present study.

An increased level of *Anaplasma* recorded in this study i.e., 48% and 15% in NWZ and CDZ respectively, could be associated with persistence

of infection for life in recovered animals as reported by Eriks *et al.* (1993), besides it can be transmitted by various species of ticks, biting flies and also iatrogenic transmission (Kumar *et al.*, 2015). The findings of the present study are in corroboration with observation of Elhariri *et al.* (2017) who reported that 69.3% of the blood samples collected from the Egyptian buffaloes were found positive for *Anaplasma marginale* when *mspla* gene was amplified. In this study, the percentage of infection of *Theileria* and *Anaplasma* was found to be high in NWZ than CDZ. This observation is akin to the findings of Kolte *et al.* (2017) who recorded a significantly higher rate of infection in rainfall scarcity zone than moderate rainfall zone. The annual rainfall of NWZ is lower than CDZ, and hence, it is presumed that the prevalence of haemoprotozoan diseases could be influenced by the rainfall rate and relative humidity prevailing in a particular locality as hot and humid climate is an essential factor for survival of tick vectors.

In this study, mixed infection of *Theileria* and *Anaplasma* was recorded in 5.3% of the samples collected. This is not in consonant with Khan *et al.* (2004) who recorded mixed infection of haemoprotozoan parasites in 16% of samples screened. Similarly, Malyar and Farid (2019) observed a mixed infection in 16.4% of samples from buffaloes. The variation in the rate of infection could be due to climatic condition and abundance of vectors.

In the present study, high prevalence of *Theileria* sp. and *Anaplasma* sp. was recorded in animals of 1 year followed by 2 year and above 4 years old, but statistically not significant ($P < 0.05$) (Figure 4). Interestingly, the rate of infection among the animals of 3 years old was comparatively lower than other age groups. The high prevalence of infection among 1 to 2 years could be due to waning

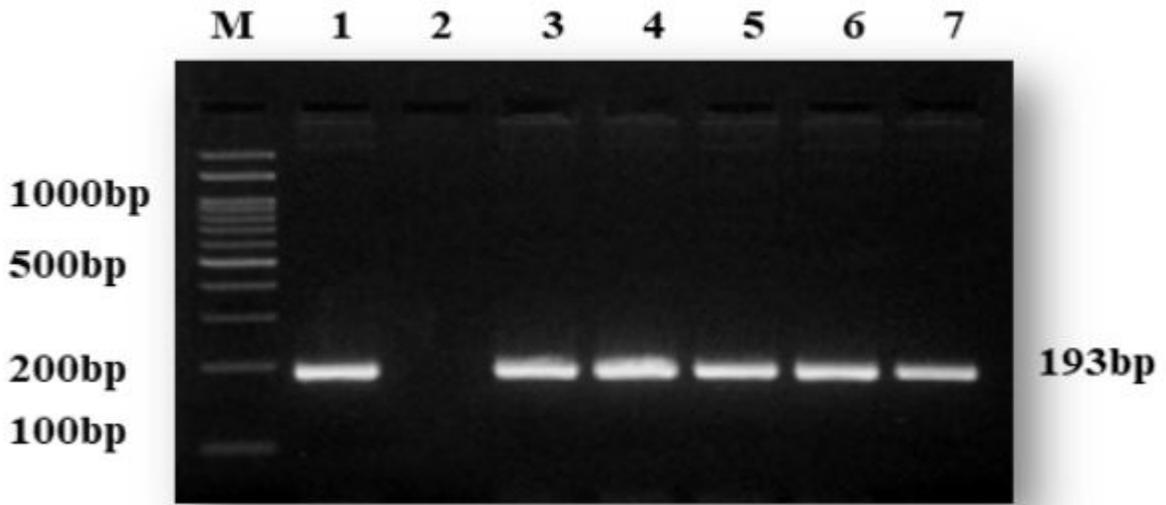


Figure 1. PCR amplified of 193 pb of *Theileria* sp. M- DNA marker.

Lane 1: Positive control;

Lane 2: Negative control;

Lane 3-7: Positive field samples.

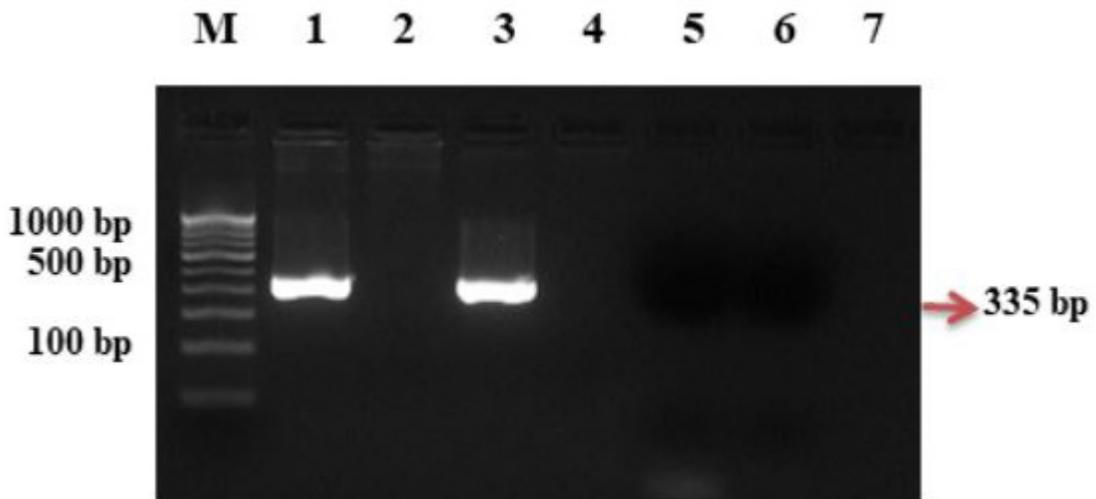


Figure 2. PCR amplified of 335 pb of *Anaplasma* sp. M- DNA marker.

Lane 1: Positive control;

Lane 2: Negative control;

Lane 3: Positive field sample;

Lane 4-7: Negative field samples.

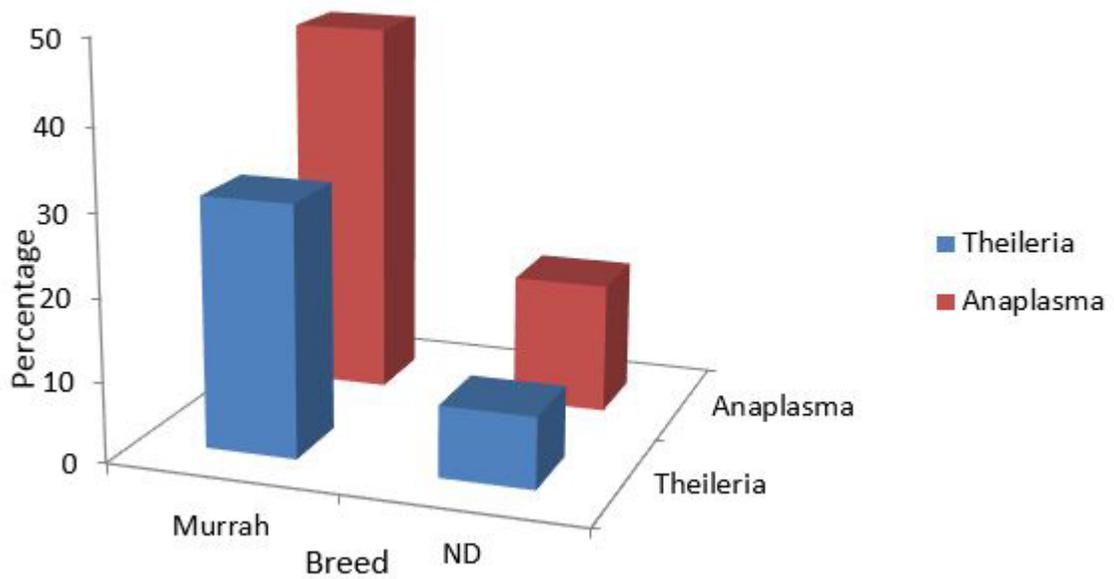


Figure 3. Carrier status of *Theileria* and *Anaplasma* in different breeds.

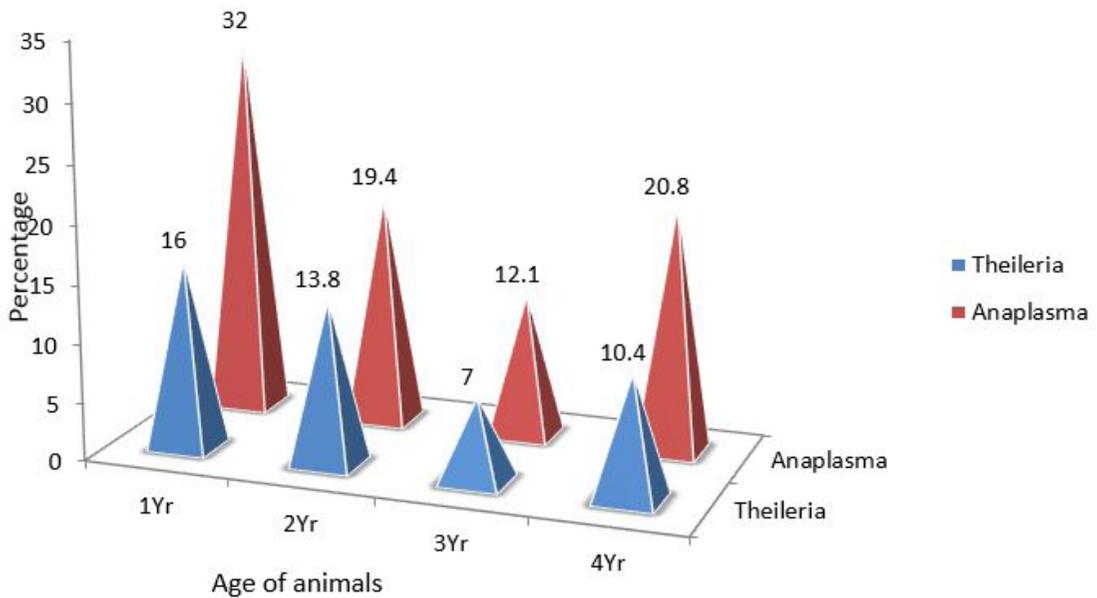


Figure 4. Carrier status of *Theileria* and *Anaplasma* in different age of animals.

of maternal immunity, which usually confers protection against haemoprotozoan diseases in young animals for 3 months (Yeruham *et al.*, 1995). But in adults, the physiological factors such as oestrus, pregnancy and lactation induced transient immunosuppression might be the reason for high infection rates as suggested by Durani (2003). The findings of the present study are not in accordance with observations of Utech and Wharton (1982) who reported that young cows were more resistant than older cows; this difference could be due to species variation.

ACKNOWLEDGEMENTS

Funding for the project was provided through the Farmed Animal Diseases and Health by Department of Biotechnology, Government of India under Indo - UK Collaboration (BBSRC). The authors would like to thank Veterinarians for help in sample collection and thank the Dean, Veterinary College and Research Institute, Namakkal for facilities provided.

REFERENCES

- Durani, A.Z. 2003. *Epidemiology, serodiagnosis and chemotheraphylaxis of theileriosis in cattle*. University of Veterinary and Animal Sciences, Lahore, Pakistan.
- Elhariri, M.D., R.A. Elhelw, D.A. Hamza and D.E. Soliman. 2017. Molecular detection of *Anaplasma marginale* in the Egyptian water buffaloes (*Bubalus bubalis*) based on Major surface protein 1 α . *Journal of the Egyptian Society of Parasitology*, **47**(2): 247-252. DOI: 10.21608/JESP.2017.77758
- Eriks, I.S., D. Stiller and G. Palmer. 1993. Impact of persistent *Anaplasma marginale* rickettsemia on tick infection and transmission. *J. Clin. Microbiol.*, **31**(8): 2091-2096. DOI: 10.1128/jcm.31.8.2091-2096.1993
- Khan, M.Q., A. Zahoor, M. Jahangir and M.A. Mirza. 2004. Prevalence of blood parasites in cattle and buffaloes. *Pak. Vet. J.*, **24**(4): 193-195. Available on: http://www.pvj.com.pk/pdf-files/24_4/193-195.pdf
- Kolte, S.W., S.D. Larcombe, G.S. Jadhao, S.P. Magar, G. Wathi, N.V. Kurkure, E.J. Glass and R.B. Shiels. 2017. PCR diagnosis of tick-borne pathogens in Maharashtra state, India indicates fitness cost associated with carrier infections is greater for crossbreed than native cattle breeds. *PLoS ONE*, **12**(3). DOI: 10.1371/journal.pone.0174595
- Kumar, T., N. Sindhu, G. Charaya, A. Kumar, P. Kumar and G. Chandratere. 2015. Emerging status of anaplasmosis in cattle in Hisar. *Vet. World*, **8**(6): 768-771. DOI: 10.14202/vetworld.2015.768-771
- Kundave, V.R., A.K. Patel, P.V. Patel, J.J. Hasnani and C.G. Joshi. 2015. Detection of theileriosis in cattle and buffaloes by polymerase chain reaction. *Journal of Parasitic Diseases*, **39**(3): 508-513. DOI: 10.1007/s12639-013-0386-2
- Lalchandhi, C.L. 2001. Efficacy of various drugs against blood protozoa in Kundhi buffaloes. *Parasitologia*, **32**: 165-176.
- Malyar, R.M. and R.A. Farid. 2019. Incidence of haemoprotozoan diseases in cattle & buffaloes Bhisood district Nangarhar province. *Journal of Agriculture and Veterinary Science*, **12**(1): 48-51. Available on: <https://www.iosrjournals.org/iosr-javs/>

papers/Vol12-issue1/Series-3/H1201034851.pdf

Resali, D.P. 2000. Recent trends in buffalo production in Nepal: A review, Buffalo Newsletter. *Bulletin of The FAO Inter-Regional Cooperative Research Network on Buffalo*, Europe-Near East. **14**: 6-10.

Yeruham, I., A. Hadani, F. Galker and S. Rosen. 1995. A study of an enzootic focus of sheep babesiosis (*Babesia ovis*, babes, 1892). *Vet. Parasitol.*, **60**(3-4): 349-354. DOI: 10.1016/0304-4017(95)00783-7