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MOLECULAR IDENTIFICATION OF THEILERIOSIS IN GOATS OF KERALA

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Abstract: A PCR-RFLP protocol targeting 18S ribosomal RNA was utilised to identify *Theileria/ Babesia*spp among naturally infected goats of Kerala. Among the apparently healthy adult animalssubjected to the study, the protocol could detect theileriosis in 64 per cent goats while blood smear examination revealed piroplasm in only 18 per cent samples. None of the goats harboured *Babesia* spp. The results of the study highlighted the enhanced sensitivity of PCR-RFLP protocol for providing molecular evidence of subclinical theileriosis and stressed the need to investigate the species spectrum of *Theileria* and the host resistance status for the infection.

Keywords: Goats,Kerala, PCR-RFLP, *Theileria* spp.

Introduction

Theileriosis and babesiosisare the most economically important protozoan infectionwith a major impact on small ruminant production in tropical countries. Babesiosis in small ruminants is caused by *Babesiaovis*, *B.motasi* and *B. crassa*, whereas, theileriosis is caused by at least six different species of *Theileria*, of which *T. lestoquardi* (*hirci*) is highly pathogenic and *T. ovis* is mildly pathogenic (Aktas *et al.*, 2005).Acute piroplasmosis in goats is often detected by microscopic examination of Giemsa-stained peripheral blood smears. *Theileria* spp. is a small, highly pleomorphic piroplasm, accurate detection of which demands considerable technical expertise, especially due to the small size of caprine RBC's (Li *et al*., 2014). *Babesia* spp. also occurs in the erythrocytes and causes infection clinically similar to theileriosis. Moreover, routine microscopy fails to detect subclinical infections which develop after the acute phase. Hence, a sensitive and specific diagnostic tool for accurate detection and differentiation of piroplasm is required. Nested PCR (Atlay *et al*., 2005), reverse line blot hybridisation (Atlay *et al*.,2007), PCR (Durrani *et al*., 2011; Iqbal*et al*., 2011; Shahzad *et al*., 2013) and PCR-RFLP (Razmi and Yaghfoor, 2013; Hegab *et al*., 2016) were utilised for detection of ovine theileriosis in sheep in different countries. Reports from India, based on

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the results of blood smear examination, indicated that theileriosis was prevalent in small ruminants of different states including Karnataka (Harish *et al*., 2006), Kerala (Anumol *et al*., 2011), Arunachal Pradesh (Tayo *et al*., 2011), Tamil Nadu (Velusamy *et al*., 2015), Madhya Pradesh (Sahu*et al*., 2016) and Tripura (Das, 2017). However, the presence of tick transmitted intraerythrocyticpiroplasms in goats of South India is neither identified nor characterised by molecular methods. A PCR protocol to detect and differentiate *Theileria* and *Babesia* spp. in goats would serve to detect the infection timely and to initiate accurate treatment protocols. Hence, with this objective the study was undertaken for standardising a molecular tool to detect theileriosis and babesiosis in Kerala, South India and to assess the carrier status for these pathogens among goats.

Materials and methods

Sample collection: Whole blood samples of goats (n=50) were collected from different places of Kerala *viz*., Thrissur, Ernakulam, Palakkad and Wayanad. The goats selected for the study were apparently healthy adult stock belonging to Malabari or Malabari cross breeds. Thin peripheral blood smears were also simultaneously collected and subjected to Giemsa's staining and observed under the microscope for morphological identification.

PCR protocol: DNA isolation was done from whole blood using phenol- chloroform method (Sambrookand Russell, 2006).Specific primers targeting a region of 18S ribosomal RNA gene of the piroplasms (TF15′-GCATTCGTATTTAACTGTCAGAGG-3′ and TR15′ GATAAGGTTCACAAAACTTCCCTAG-3′) were selected according to Jalali*et al*, (2013). Later, PCR was done in a 12µLreaction mix containing 6.5 µL of master mix(SRL, India), 12.5 pmol each of forward and reverse primers, 1 µL of template DNA and 4 µL of nuclease free water. The amplification was performed in a gradient thermocycler (Biorad, USA) with the following program: an initial denaturation step at 94°C for 5 min followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 56-60°C for 60 s and extension at 72°C for 60 s with a final extension step of 72°C for 5 min.A non-template control was included in each run. The amplicons were electrophoresed on 1.5% agarose gel and viewed in gel doc (Biorad, USA).The PCR amplified DNA products were sequenced (SciGenom Labs Pvt Ltd, Kochi).

RFLP protocol: Restriction fragment length polymorphism (RFLP) of PCR products of the 18S rRNA gene was done with the enzyme *HindII,* to detect the presence of restriction sites that would confirm the genus identity and differentiate between *Theileria* and *Babesia spp.* The enzymatic digestion was carried out in 20 µl reaction mixture consisting of 10 µl PCR amplicons, 2 µl of 10X corresponding buffer, and 0.1 µl (1 U) restriction enzyme and

nuclease free water. The digestion mixture was incubated at 37°C for one hour. Electrophoresis was carried out using 12 per cent polyacrylamide gel and analysed.

Results and discussion

Out of the total50samples subjected to the study, nine blood smears were positive for piroplams of *Theileria* spp. Thirty two samples revealed a PCR product of 872bp size (Fig 1) which was sequenced bidirectionally. The aligned sequence upon analysis by BLASTn tool (*https://blast.ncbi.nlm.nih.gov*) revealed 99% similarity to several published sequences of corresponding gene of *Theileria* spp. (KF597086.1, KF597085.1, KF597080.1, KF597079.1, AB602881.1, AB602878.1, AB602877.1, AB012201.1, AB012200.1, KF597087.1). Further, PCR- RFLP with *HindII* yielded two bands of approximately 449bp and 423bp sizes (Fig 2), which proved that all positive samples were *Theileria* spp. as per Jalali *et al.*, (2013) and Zaeemi *et al.*, (2011).

It is noteworthy that while piroplasmosis could be detected in only18 percent goats by light microscopy, PCR-RFLP could identify the infection in 64 per cent animals suggesting the high sensitivity of the protocol. Lack of sensitivity of conventional microscopy in detection ovine theileriosis was reported by Atlay*et al.* (2005), Razmi *et al*. (2013), Shahzad *et al.* (2013) and Hegab *et al*. (2016). None of the goats were found infected with *Babesia* spp. Anumol (2011) could identify four cases of *Babesia* spp out of 250 anaemic goats in Kerala. The PCR-RFLP protocol proved to very accurate to detect *Theileria* species which otherwise could not be reliably detected and differentiated according to their morphology by microscopy, especially in subclinical infections.

A high prevalence of theileriosis as detected by PCR-RFLP in apparently healthy animals signals the presence of subclinical infection or the existence of non-pathogenic species which needs to be ruled out further. Studies are to be carried out to differentiate the various species of *Theileria* prevalent in goat population of the State. The possibility of the Malabari and Malabari cross breeds to withstand the clinical effects of theileriosis also warrants detailed investigation.

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References

[1] Aktas, M., Altay, K. and Dumanli, N. 2005.Survey of *Theileria* parasites of sheep in eastern Turkey using polymerase chain reaction. *Small Rum Res*. 60: 289–293.

[2] Altay, K., Dumanli, N., Holman, P.J. and Aktas, M. 2005. Detection of *Theileria ovis* in naturally infected sheep by nested PCR. *Vet parasitol*. 127: 99-104.

[3] Altay, K., Dumanli, N. and Aktas, M. 2007. Molecular identification, genetic diversity and distribution of *Theileria* and *Babesia* species infecting small ruminants.*Vet parasitol*. 147: 161-165.

[4] Anumol, J., Tresamol, P. V., Saranya, M.G., Vijaykumar, K. and Saseendranath, M.R.A study on aetiology of anaemia in goats.*J. Vet. Anim. Sci*. 42: 61-63.

[5] Das, S.S. 2017. Prevalence of haemoprotozoan infections in domestic animals in Tripura (India).*J. Parasitol. Appl. Anim. Biol*. 21: 53-56.

[6] Durrani, A.Z., Younus, M., Kamal, N., Mehmood, N. and Shakoori, A.R. 2011. Prevalence of ovine *Theileria* species in district Lahore, Pakistan.*Pak. J. Zool*. 43: 57-60

[7] Harish, B.R., Chandranaik, B.M., Renukaprasad, C., Jayakumar, S.R. and Krishnappa, G. 2006. Incidence of haemoprotozoan diseases in Karnataka.*Indian J. Vet. Med.* 26: 30-31.

[8] Hegab, A., Fahmy, M.M., Mahdy, O. and Wahba, A.A. 2016. Parasitological and molecular identification of *Theileria* species by PCR-RFLP method in sheep, Egypt.*Int. J. Adv. Res. Biol. Sci*. 3: 48-55.

[9] Iqbal, F., Ali, M., Fatima, M., Shahnawaz, S., Zulifkar, S., Fatima, R., Shaikh, R.S., Shaikh, A.S., Aktas, M. and Ali, M. 2011. A study of prevalence and determination of the risk factors of infection with *Babesia ovis* in small ruminants from southern Punjab (Pakistan) by PCR amplification.*Parasite*.18: 229-234

[10] Jalali, S., Khaki, Z., Kazemi, B., Rahbari, S., Shayan, P., Bandehpour, M. and Yasini, S. 2013.Molecular Detection and Identification of Theileria Species by PCR-RFLP Method in Sheep from Ahvaz, Southern Iran. *Iranian.J. Parasitol.*9: 99-106.

[11] Li, Y., Zhang, X., Liu, Z., Chen, Z., Yang, J., He, H., Guan, G., Liu, A., Ren, Q., Niu, Q., Liu, J. and Yin, H. 2014.An epidemiological survey of *Theileria* infections in small ruminants in Central China. *Vet Parasitol.* 200:198-202.

[12] Razmi, G. and Yaghfoori, S. 2013. Molecular surveillance of *Theileria ovis*, *Theileria lestoquardi* and *Theileria annulata* infection in sheep and Ixodid ticks in Iran. *Onderstepoort. J. Vet. Res*. 80: 635-640.

[13] Razmi, G., Yaghfoori, S. and Heidarpour, M. 2013. Molecular detection of Theileria spp. in sheep and vector ticks in Fasa and Kazeroun areas, Fars Province, Iran. *Arch. Razi. Inst*. 68: 159-164.

[14] Sahu, P., Gaherwal, S., Kanhere, R.R., Shrivastava, C.S. and Aziz, R.U. 2016. Prevalence and incidence of ticks in Goat (Capra Hircus) in Mhow(M.P.). *Ind. J. Appl. Res*. 6: 42-44.

[15] Sambrook, J. and Russell, D.W. 2006. Purification of nucleic acids by extraction with phenol: chloroform.2006. *Cold Spring Harbor Protocols*. 1: 4455.

[16] Shahzad, H., Noor, H., Ahmad, M., Munir, R., Saghar, M., Mushtaq, M., Ahmad, N., Akbar, G. and Mehmood, F. 2013. Prevalence and molecular diagnosis of *Babesia ovis* and *Theileria ovis* in Lohi sheep at livestock experiment station (LES), Bahadurnagar, Okara, Pakistan. *Iranian. J. Parasitol*. 8: 570-578.

[17] Tayo, T., Longjam, N. and Perme, B. 2011. Protozoan diseases of livestock in Arunachal Pradesh- An overview. *Vet. Wld*. 4: 332-336.

[18] Velusamy, R., Rani, N., Ponnudurai, G. and Anbarasi, P. 2015. Prevalence of intestinal and haemoprotozoan parasites of small ruminants in Tamil Nadu, India.*Vet. Wld*. 8: 1205-1209.

[19] Zaeemi, M., Haddadzadeh, H., Khazraiinia, P., Kazemi, B. andBandehpour, M. 2011. Identification of different *Theileria*species (*Theilerialestoquardi*, *Theileriaovis*, and *Theileriaannulata*) in naturally infected sheep using nested PCR–RFLP. *Parasitol Res*.8:837-843.

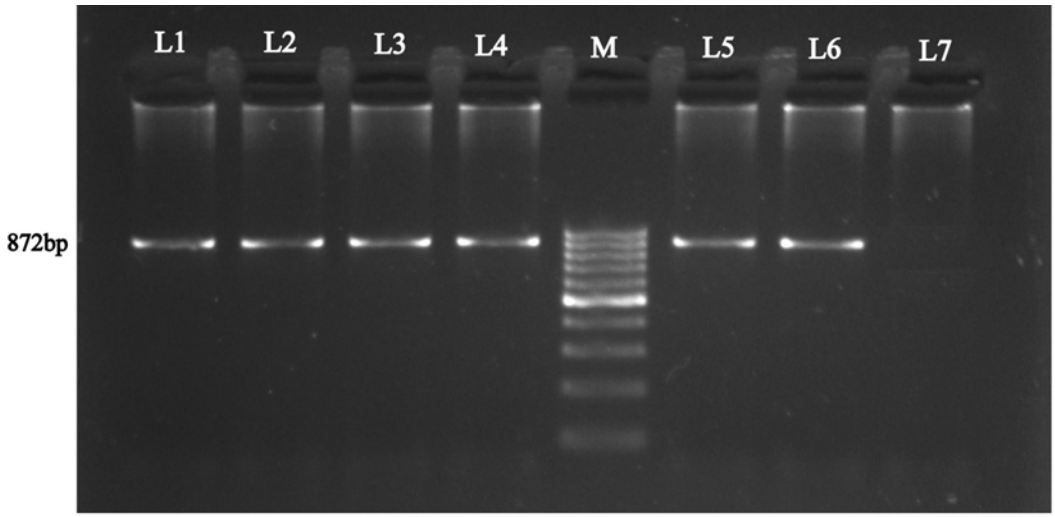

Fig 1: PCR amplicons of *Theileria* spp

Lanes: L1, L2, L3, L4, L5, L6- Amplicons of *Theileria* spp.
L7- No template control
M : 100 bp ladder

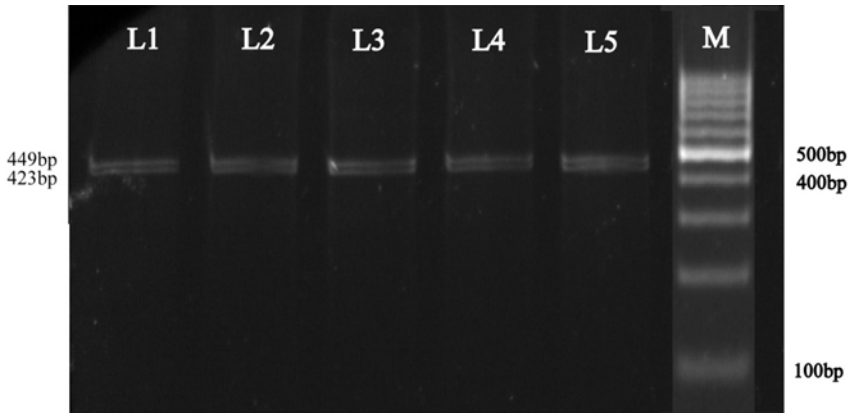

Fig 2: *Hind II* digested products of *Theileria* spp.

Lanes- L1, L2, L3, L4, L5 *Hind II* digested products
M- 100 bp ladder