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ORIGINAL ARTICLE **OPEN ACCESS**

Epidemiological Study to Determine the Prevalence of *Theileria annulata* Infection in Cattle-calves using PCR for Targeting Tams-1 Gene

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ABSTRACT
A total of 100 blood samples were collected in ethylene diamine tetra acetic acid (EDTA @ 1 mg/ml) vacutainer from cattle-calves irrespective of their age, sex and breed brought to Teaching Veterinary Clinical Complex of College of Veterinary and Animal Science, Bikaner for treatment. Genomic DNA were isolated from whole blood using QIAamp® DNA blood mini kit (QIAGEN, GmbH, Germany) as per protocol and subjected to polymerase chain reaction (PCR). By PCR, 41 samples were found positive for Theileria annulata infection which produced 721-bp amplicon in 1 per cent agarose gel.
Key words: *Genomic DNA, PCR, Theileria annulata, amplicon, agarose gel*

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INTRODUCTION

Theileriosis, caused by various intra-erythrocytic protozoan parasites of the genus *Theileria,* is a tick borne disease of domestic and wild animals. *Theileria* parasites enter the bovine host during tick feeding as sporozoites, which rapidly invade mononuclear leukocytes [6]. It causes significant economic losses in large parts of Asia [10]. It is mainly seen in cattle, sheep and goat as well as in wild and captive ungulates [12]. This intracellular infection inflicts economic burden on cattle breeders in terms of mortality and morbidity as well as expenses spent on prophylactic measures against disease and treatment [7].

Theileria spp. infection can cause acute, subacute or chronic disease pathology [8]. In *T. annulata* infection, the most common clinical symptoms are weakness, weight loss, anorexia, high body temperature, petechia on the conjunctival mucosa, swollen lymph nodes, anaemia and cough. On later stages of theileriosis, infected animals cannot stand up, their body temperatures are under normal values (< 38.5°C), and icterus, dehydration and blood in faeces are the occasional clinical symptoms [4,12]. The prevalence of *T. annulata* infection is 41% by nPCR for small sub-unit ribosomal RNA (SSU-rRNA) gene [9].

Molecular identification provides two primary advantages to phenotypic identification; it is more rapid turnaround time, and improved accuracy of identification [1]. Advances in molecular diagnostic techniques have resulted in the improved detection, identification and genetic characterization of many haemoparasites. Species-specific polymerase chain reaction (PCR) has been developed for the detection and identification of various *Theileria* species and has been shown to have higher sensitivity and specificity compared with serological assays and examination of Giemsa stained blood smears [5, 13]. Thus PCR is thought to be more practical and widely implemented technique not only for diagnosis but also for epidemiological investigation of haemoprotozoa of dairy animals primarily in developing countries.

MATERIALS AND METHODS

In the present study, one hundred cattle-calves irrespective of their age, sex and breed brought to Teaching Veterinary Clinical Complex of College of Veterinary and Animal Science, Bikaner for treatment were screened for bovine tropical theileriosis. After clinical examination of cattle-calves, 2.0 ml of blood sample was collected from Jugular vein of each cattle-calf suspected for bovine tropical theileriosis aseptically in ethylene diamine tetra acetic acid (EDTA @ 1 mg/ml) vacutainer for molecular analysis and stored at -20°C until DNA extraction.

Genomic DNA was isolated from whole blood using QIAamp® DNA blood mini kit (QIAGEN, GmbH, Germany) as per protocol. Aliquots of extracted genomic DNA were kept at -20°C. PCR was performed using *Theileria annulata* specific primers (Table 1) for targetting the 30-kDa major *T. annulata* merozoite surface antigen (Tams-1 gene) in a final reaction volume of 25 µl (Table 2).

Table 1: Primers used in the PCR

Primers	Sequence	Amplified DNA fragme (bp)
N516 (F)	GTA ACC TTT AAA AAC GT	721
N517 (R)	GTT ACG AAC ATG GGT TT	

Table 2: Composition of PCR master mix

Reagent	Volume
CoralLoad PCR buffer	5.00µl
25 mM $MgCl_2$	3.00 µl
10 mM dNTP mix	1.00 µl
Taq DNA polymerase	0.25 µl
20pmol forward primer-N516	0.75 µl
20 pmol reverse primer-N517	0.75 µl
Template DNA isolated from the blood sample	3.00 µl
Nuclease free water	11.25 µl
Total	25.00 µl

The thermo cycle profile was as described below:

1. Initial denaturation at 94°C for 3 minutes.
2. Then denaturation at 94°C for 1 minute for 30 cycles.
3. Annealing at 55°C for 1 minute for 30 cycles.
4. Extension at 72°C for 1 minute for 30 cycles.
5. Final extension at 72°C for 3 minutes.
6. Holding at 4°C until the samples were taken out from thermal cycler.

These PCR products were checked by electrophoresis on 1 per cent agarose gel and visualized using a gel documentation system (Syngene, UK).

RESULT AND DISCUSSION

Out of 100 DNA extracted from blood samples of cattle-calves, only 41 samples were found positive for *Theileria annulata* which produced 721-bp amplicon in 1 per cent agarose gel (Fig.1).

Microscopic detection of the piroplasms and schizonts in the blood smears and/or lymph node aspirate smears from the suspected host is the true 'gold standard' diagnostic test available for theileriosis, along with the clinical signs of the disease, such as high fever and enlargement of lymph nodes also help in diagnosis. These tests are robust, but have poor sensitivity and higher dependency on the qualified laboratory technicians. The low level of parasitemia in the case of carrier animals and chronic cases holds importance as they make the animal a reservoir of infection for the whole herd. PCR assays permit identification of parasite at levels far below the detection limit of the commonly used parasitological techniques and has superiority in separating clinical and subclinical forms of parasitic infection.

Thus, PCR is preferred method for diagnosis of theileriosis because this method is more sensitive and specific than other conventional methods [2, 3, 11].

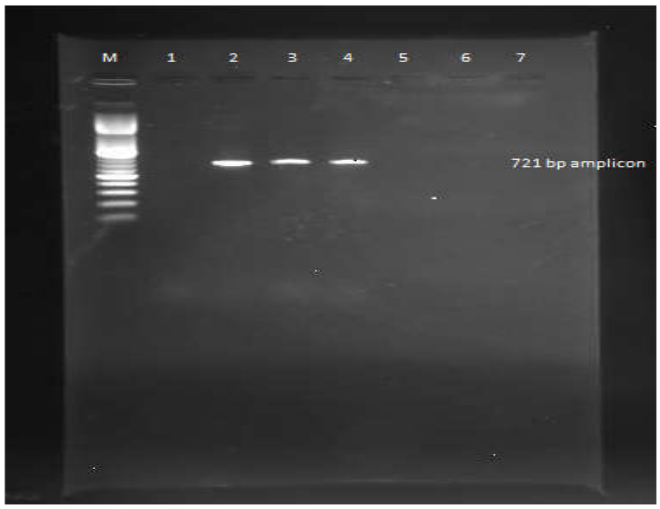

Fig.-1: Photograph showing 721-bp amplicons (*Theileria annulata* species specific) which subjected to electrophoresis on 1% agarose gel and stained with ethidium bromide.
Lane M: 100-bp Generuler™ DNA ladder
Lane 1: Negative control
Lane 2: Positive control
Lanes 3-7: Clinically suspected samples
Lanes 3 & 4: Positive samples.

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