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Short Communication

DETECTION OF CUTANEOUS FORM OF BOVINE TROPICAL THEILERIOSIS IN CROSSBRED COWS IN TAMILNADU, INDIA

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ABSTRACT: Bovine theileriosis is one of the most important vector-borne haemoprotozoan diseases and causes huge economic losses in cross-bred animals, particularly in tropical countries like India. The present study describes cutaneous theileriosis in two cross-bred cows presented to a veterinary hospital, VCRI, Namakkal, with a history of fever, anorexia, and reduced milk yield. Clinical examination of animals revealed ocular edema with pale conjunctival mucous membrane, mild enlargement of the prescapular lymph nodes, and numerous skin nodules all over the body. Microscopic examination of the skin nodules revealed the presence of schizonts (Koch's Blue Bodies) in lymphoblasts. Peripheral blood smear examination showed mild parasitaemia in erythrocytes, and further confirmation by PCR using *Theileria-specific* primers showed the desired 1098bp amplification comparable to positive control. Appropriate treatment led to the recovery of the animals with the total disappearance of the skin lesions.

Key words: Cross-bred cows, Cutaneous theileriosis, Skin nodules, Schizonts, *Theileria* sp., PCR.

Bovine theileriosis poses a major threat to animal health and production in India where hot and humid climates are prevalent all year, favoring ticks propagation. It causes substantial economic loss due to decreased weight gain, drops in milk yield, abortions, and sometimes death in animals if untreated. Annual losses in the livestock industry due to tropical theileriosis alone were estimated to be US$ 384.3 million (Minjauw and McLeod 2003). It is mainly caused by intracellular protozoa, *Theileria annulata,* and transmitted by tick vector, *Hyalomma anatolicum* (Blewett and Branagan 1973, Sangwan *et al*. 1986, Das and Ray 2003). The animals usually get the infection during tick feeding through sporozoites, followed by maturation of schizonts into merozoites and subsequent infection of erythrocytes with the formation of piroplasms. Generally, animals that survive acute infection after treatment become carriers of the parasites and serve as reservoirs of infection to susceptible animals by ticks (Bishop *et al.*2004). Gharbi *et al.* (2017) described two clinical cases of tropical theileriosis in northern Tunisia with the uncommon sign of skin nodules estimated at 15–20 per 10 cm^2 and distributed all over the animal's body with 0.5–2 cm in diameter. Atypical theileriosis with cutaneous involvement in cross-bred cows naturally infected with *T. annulata* was reported in India (Narang *et al*. 2019, Geetha and Selvraju 2021).

The study

Two crossbred cows (1 Jersey cross and 1 HF cross) were presented in April, 2020 to the Veterinary College and Research Institute (VCRI) Hospital, Namakkal, Tamil Nadu, with a history of fever, anorexia, reduced milk yield and presence of skin nodules on the body without tick infestation. Upon clinical examination, no ticks were detected on the animal's body.

Blood smear examination

The blood smears were made from the ear tip for the presence of any piroplasms in red blood cells. In addition, fine-needle aspiration biopsy (FNAB) samples were taken from the swollen pre-scapular and subcutaneous nodules, and smears were prepared and stained with Giemsa stain and examined under a light microscope (100 X oil immersions).

Polymerase Chain Reaction (PCR)

A total of 2 ml of blood was collected aseptically from the jugular vein of the affected animals and genomic DNA

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was extracted from 200ul of blood using a QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol. The extracted DNA was then subjected to PCR using *Theileria-specific* primers, Forward: 5'AGTTTCTGACCTATCAG 3' and Reverse: 5'TTGCCTTAAACTTCCTTG3' (d'Oliviera *et al.*1995). Amplification was performed in a thermal cycler (Eppendorf) under the following conditions: 94°C for 3 min (initial denaturation) followed by 34 cycles of 94°C, 30 sec (denaturation), 52°C, 30 sec (annealing), 72°C, 45 sec (extension) and a final extension of 72°C for 5 min. The positive and negative control was run along with the test samples. The amplification products were subjected to electrophoresis on 1% agarose gel. The length of the amplified product was estimated using a standard 100 bp DNA marker (HiMedia, India) and the amplified products were visualized with a UV transilluminator (Bio-Rad, Gel Doc TMXR+, Molecular Imager, USA).

Results and discussion

Upon clinical examination, animals showed normal body temperature and mild to moderate enlargement of prescapular lymph nodes. Numerous skin nodules of varying sizes were prominent, particularly on the neck and shoulders, and extending to other body parts. The density of skin nodules was estimated at 10 – 20 per 10 cm^2 and 0.4 - 1.2 cm in diameter (Fig.1). Clinically, ocular oedema with pale conjunctival mucous membrane was observed (Fig. 2). Giemsa-stained peripheral blood smear showed the presence of *Theileria* piroplasms in erythrocytes with mild parasitaemia (Fig. 3). The microscopic examination of the Giemsa-stained smear from swollen lymph nodes showed the presence of KBB in lymphoblast cells, and smear from skin nodules showed the presence of schizont in lymphoblast cells as well as free schizont outside the cells (Fig. 4). The polymerase chain reaction using *Theileria* specific primers revealed the amplification of the desired product size of 1098 bp specific for *Theileria* species was observed, which was comparable to the positive control (Fig. 5).

The infected animals were treated with buparvoquone at 2.5 mg/kg BWT (ButalexTM, MSD animal health) along with oxytetracycline at 10 mg/kg BWT and supportive therapy (B complex liver extract + vitamin B12 injection (Zydus Animal Health). After three weeks of treatment, the owner reported a gradual improvement in the condition of the animals with regression of the skin nodules, and the milk yield had returned almost to the normal level.

Tropical bovine theileriosis occasionally appears after the acute phase (Tsur-Tchernomoretz *et al.*1960). Indeed, several scattered publications have been reported as cutaneous signs due to tropical theileriosis (Uilenberg and Zwart 1979, Manickam *et al.* 1984). Urticarial lesions due to theileriosis have been reported in an indigenous cow, which is highly suggestive of the allergic consequence of acute parasitaemia responsible for intense pruritus in animals (Sudhan *et al.*1992). Narang *et al.*(2019) reported a rare case of non-fatal theileriosis with multiple subcutaneous nodular masses (2 - 4 cm) on the neck and abdomen in a cross-bred cow in Punjab. In our study, two cases of cutaneous expression of theileriosis were observed in crossbred cows in Tamil Nadu, India, and the density of nodules was estimated at 10 – 20 per 10 cm^2 and 0.4 – 1.2 cm in diameter. Similarly, Gharbi *et al.* (2017) also described two clinical cases of tropical theileriosis in northern Tunisia with the uncommon sign of skin nodules estimated at 15–20 per 10 cm^2 and distributed all over the animal's body with 0.5–2 cm in diameter. The development of skin nodules in the current study may be due to the metastasis of schizont-infected cells from prescapular lymph nodes to the non-lymphoid organs

Fig. 1. Numerous skin nodules on the neck, shoulders and body of a Jersey cow (A) and a HF cow (B), infected with cutaneous theileriosis

Fig. 2. Ocular oedema with pale conjunctival mucous membrane in a cow infected with cutaneous theileriosis.

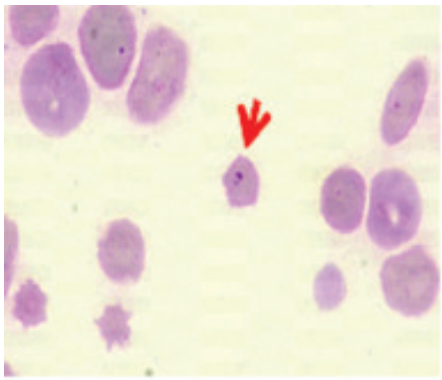

Fig. 3. Microscopic Examination of Giemsa stained blood smear showing piroplasm of *Theileria* parasite in red blood cell.

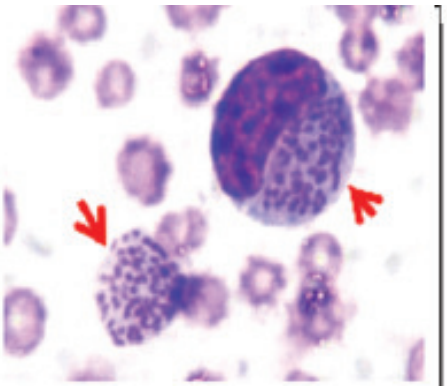

Fig. 4. Fine-needle aspiration of skin nodular biopsy sample showing presence of schizont in lymphoblast as well as outside the cell.

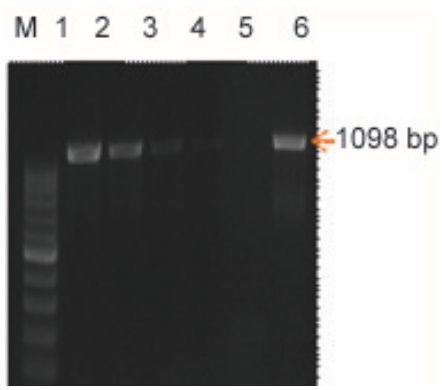

Fig.5 Agarose gel electrophoresis showing amplified 1098-bp fragment of *Theileria* DNA produced by PCR. L: 100 bp DNA ladder, L: 1 to 4 - Samples positive for *Theileria* DNA, L: 5 Non-template control, L: 6 Positive control.

including the skin of adult animals. The possible mechanism for metastasis of schizonts may be due to the circulating phagocytes from lymph nodes to the skin. In contrast to our observation of skin nodules in adult animals, there have been some reports showing widespread skin nodules only in calves infected with *T.annulata* (Shastri *et al*. 1982, Gupta *et al*. 2004, Naik *et al*. 2010).

PCR has allowed the development of sensitive and specific diagnostic assays for molecular characterization of *Theileria sp.* in cattle (Tanaka *et al.*1993). It is also reflected in our study that molecular confirmation of desired product size of 1098 bp specific for *Theileria* sp. was observed which was comparable to the positive control. In the present case, it was observed that a single dose of buparvaquone is effective along with oxytetracycline and supportive therapy for three days which is also stated by Narang *et al*. (2019).

The present study reports bovine cutaneous theileriosis in crossbred cows in Tamil Nadu, India. Dermatological signs in bovine theileriosis are considered rare. The majority of these animals may die as a consequence of late diagnosis. Therefore, veterinarians should be aware of this type of cutaneous skin nodules and prompt treatment may save the animals.

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