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Short Communication

Triumphant recovery of *Theileria annulata* induced corneal opacity in a calf

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Abstract

Corneal opacity due to bovine tropical theileriosis (BTT) in 12 months old female crossbreed calf was reported in Jaipur, Rajasthan. The calf was brought to the Teaching Veterinary Clinical Complex (TVCC), PGIVER, Jaipur with a history of anorexia, impaired vision and tick infestation. On clinical examination, notable signs observed were high fever (105.8°F), slightly pale mucus membrane, cloudiness in eye and enlarged prescapular lymph nodes. Lymph node biopsy, blood smear examination and *TAMS1* gene-based PCR confirmed BTT infection in the calf. Ocular lesions, anaemia and other complications of the calf were resolved successfully with treatment of single dose of buparvaquone @ 2.5 mg/kg BW deep IM and hematinic, along with supportive therapy.

Keywords: Bovine tropical theileriosis, Buparvaquone, Corneal Opacity, Exophthalmos

Highlights

- Corneal opacity case of bovine tropical theileriosis (BTT) in a calf was reported in Jaipur.
- Lymph node biopsy, blood smear examination and *TAMS1* gene-based PCR confirmed BTT infection.
- The calf was treated successfully with buparvaquone, hematinic, and supportive therapy.

Bovine tropical theileriosis (BTT), caused by an obligate intracellular apicomplexan blood protozoan parasite *Theileria annulata*, poses major threat to dairy animals in India (Tuli *et al*., 2015). *Hyalomma anatolicum* and *Hyalomma detritum* ticks are usually implicated in the transmission of *T. annulata*. (Taylor *et al*., 2007). The most noticeable symptoms of BTT are high fever (40.5–41.5°C), laboured breathing, inappetence, cessation of rumination, enlargement of regional superficial lymph nodes, lacrimation, cachexia, conjunctivitis, anaemia and profound leucopenia (Khan *et al*., 2011; Saini *et al*., 2018). Early diagnosis and prompt treatment can limit BTT. The restricted detection limit of microscopy of Giemsa-stained blood smear, xenodiagnosis, and serological diagnosis such as complement fixation test (CFT), indirect fluorescent antibody tests, ELISA etc., necessitates the use of molecular assays such as PCR to detect a small amount of parasite DNA that is present in blood (Mans *et al*., 2015). TAMS1 is a major immune-dominant surface antigen present in the merozoites of *T. annulata* (Gubbels *et al*., 2000). TAMS1 plays an imperative role in the development of diagnostic test and recombinant subunit vaccine (Boulter and Hall, 1999). *TAMS*-1 based PCR was utilized for the diagnosis of BTT. The TAMS gene gave

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specific amplicons of size 733 bp. *T. annulata* merozoite surface antigen (TAMS) is widely used for phylogenetic analysis throughout the globe. TAMS is highly specific for *T. annulata* and does not show cross reactivity with other *Theileria* spp. like *T. parva*, *T. mutans*, *T. sergenti*, *T. buffali*, *T. velifera* and *T. taurotragi* (d'Oliveria *et al*., 1995). Although *T. annulata* induced corneal opacity cases have been documented (Joshi *et al*., 2017), corneal opacity cases are rarely encountered in regular practice (Singh *et al*., 2015). The present study reports a case of *T. annulata* induced corneal opacity in 12 months old crossbred female calf in Jaipur region.

Twelve months old crossbreed female calf was presented to the Teaching Veterinary Clinical Complex (TVCC) PGIVER, Jaipur with the complain of anorexia, impaired vision, tick infestation, prominent cloudiness in eyes, sternal recumbency and weakness for the last five days. The clinical examination revealed pyrexia (105.8°F), enlargement of prescapular lymph nodes, tachycardia (84 beats per minute) and slight pale mucus membrane. The blood profile of the calf was 9 g/dL haemoglobin and 30% PCV indicating progressive anaemia. As the clinical view pointed towards the suspicious case of haemoprotozoan infection, blood sample was collected from the ear tip of the calf and thin blood smears were prepared. The smears were air-dried, fixed with methanol, stained with 10% Giemsa stain and examined under the oil immersion objective of a microscope (Soulsby, 1982). The microscopy of Giemsa-stained blood smears revealed the presence of signet ring-shaped piroplasms in erythrocytes (Fig. 1).

Major clinical complications observed during ophthalmic examinations were profuse bilateral lacrimation, bulging eyes, supraorbital oedema, cloudy eyes, corneal opacity (Fig. 2) and impaired vision. Ticks from thigh and neck regions were collected and examined in the Division of Parasitology, PGIVER, Jaipur as per standard recommended procedures. Lymph node aspiration was also done to examine the presence or absence of Koch's blue bodies (KBB) (Fig. 3). Molecular confirmation of *T. annulata* was done by PCR targeting *TAMS1* gene in the Department of Parasitology, DUVASU, Mathura using primers and protocol indicated in Table 1 and Fig. 4.

The calf was treated with buparvoquone (Butalex, Zydus Animal Health Ltd) @ 2.5 mg/kg body weight deep IM single dose in the cervical region. The concurrent supportive therapy, i.e., Normal saline 500 mL IV, one dose of long acting oxytetracycline (Oxytetracycline-LA, Zydus Animal Health Ltd.) @ 20 mg/kg was given intramuscularly, meloxicam (Melonex, Intas Pharmaceuticals Ltd.) @ 0.5 mg/kg intramuscularly, pheniramine maleate (Avilin, MSD Animal Health, India) @ 0.5 mg/kg intramuscularly and hematinic (Feritas, Intas Pharmaceuticals Ltd.) @ 100 mg intramuscularly

Table 1. Oligonucleotide primer and thermo-cycling profile used to amplify *TAMS* 1 gene of *T. annulata*

Primer	Primer sequence	Amplicon size	Reference
TAMS 1F	5′-ATG AGG ATG AAA AGA AAA AGG AGG AAA AAA AAG ATG T-3′	733 bp	Rajendran *et al.*, 2008
TAMS 1R	5′-GCG AAG ACT GCA AGG GGG GAG AAC T-3′		

Thermal cycling profile

PCR with *TAMS*1 gene	Initial DNA denaturation	Denaturation	Annealing	Extension	Termination
	95°C, 5 min	94°C, 1 min ×30 cycles	55°C, 1 min,	72°C, 1 min	72°C, 5 min

Theileria annulata induced corneal opacity

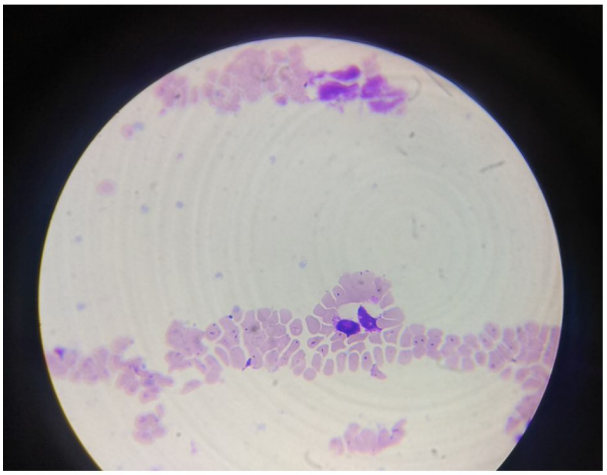


Fig. 1. Signet ring piroplasms of *Theileria annulata* in RBCs

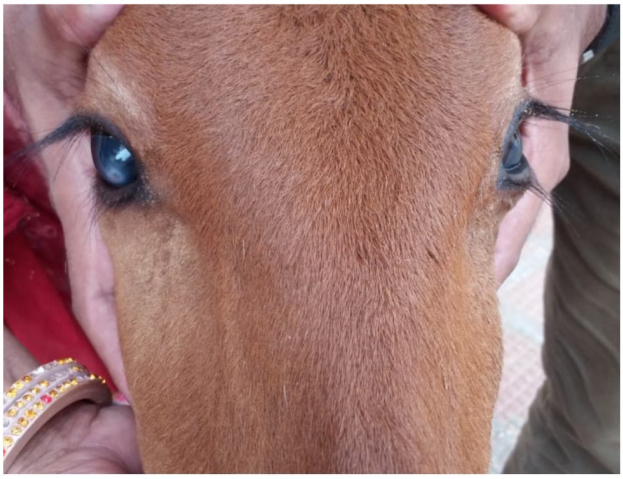


Fig. 2. Corneal opacity due to antigen-antibody complex formation

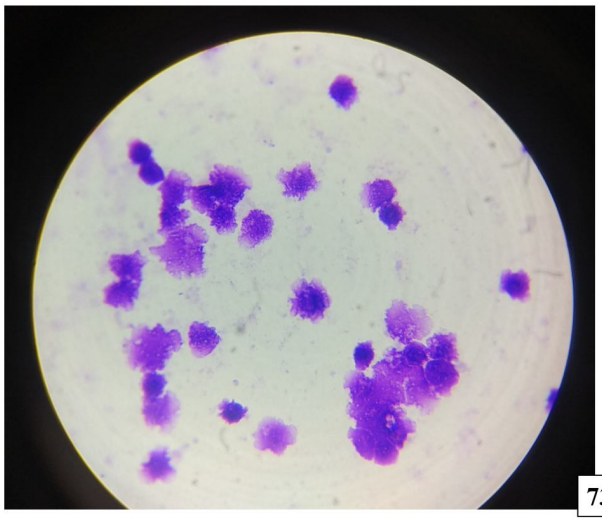
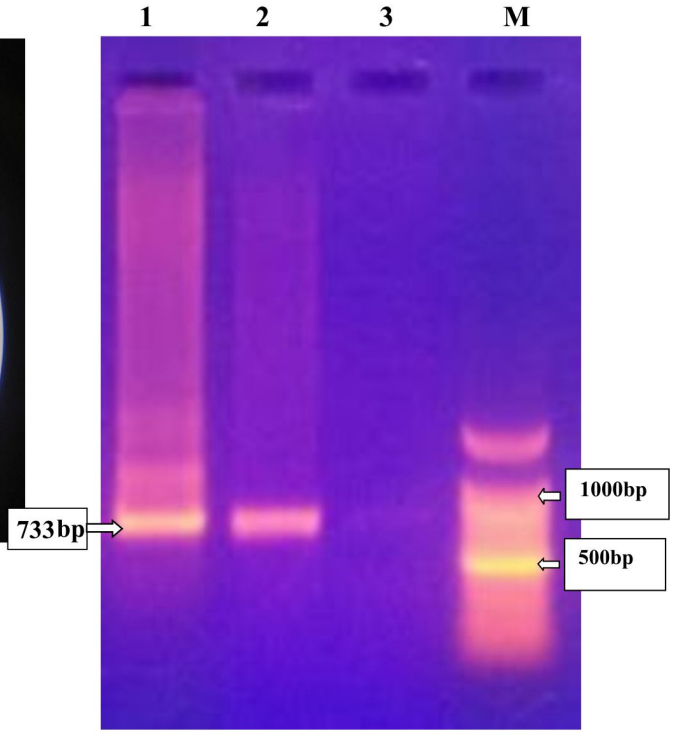


Fig. 3. Presence of Koch's blue bodies in lymphoblast of infected calf



[Lane M: 100 bp DNA marker, Lane 1: PCR amplification of a 733 bp DNA fragment of *T. annulata* (Clinical sample from calf), Lane 2: PCR amplification of a 733 bp DNA fragment of *T. annulata* (Positive Control), Lane 3: No PCR amplification (post-therapy sample from calf after recovery)]

Fig. 4. *TAMS*-1 PCR

on 0 and 7th day of therapy was also instituted. Amitraz (TAKTIC WP spray, Virbac), as per dilutions prescribed by the company, was sprayed topically in order to control ticks. The calf was also treated with 2 mL Tribivet [B1, B6, B12] intramuscularly after subsiding of fever as level of acute phase protein during fever usually remains high, and administration of Vitamin B complex also escalates acute phase protein level. Hence, Vitamin B complex is generally not indicated at the time of pyrexia. As the calf recovered, blood smears and blood samples were collected and re-screened for erythrocytic-piroplasm and Hb, PCV and *TAMS* 1 gene of merozoite of *Theileria*, respectively.

The presence of intra-erythocytic piroplasm in Giemsa-stained blood smear, KBB in lymph node smear examination directed towards theileriosis, which was in concurrence with the results of Gupta *et al*., 2004, who diagnosed theileriosis in 7 days old bovine calf on parallel ground basis. The affected calf was anaemic (Singh *et al*., 2001). The unusual Theileria induced corneal opacity cases may be due to the invasion of cornea, iris and lens by leucocytes (Irvin and Mwamachi, 1983). In early infection of theileriosis, macroschizonts multiplication occurs inside the lymphocyte and consequential into lymphoid hyperplasia and enlargement of superficial lymph nodes (Jabbar *et al*., 2008). The calf was infested with *Hyalomma* spp., which in turn suggested that *Hyalomma* was implicated in transmission of Theileria. The amplification of 733 bp TAMS1 gene confirmed the Theileria infection in calf was in accordance with the findings of Rajendran *et al*. (2008). Buparvaquone impedes the mitochondrial electron transport chain of Theileria parasite (McColm and McHardy, 1984; Hudson *et al*.,1985); that's why buparvaquone is usually indicated for effective and specific treatment of theileriosis (McColm and McHardy, 1984). The calf was recovered with aforesaid therapy, which was in accordance with previous literatures where triumphant recoveries from theileriosis infection were observed with the use of buparvaquone along with supportive therapy (Gupta *et al*., 2004; Naik *et al*., 2010; Joshi *et al*., 2017; Saini *et al*., 2018). Calf recovery from anaemia may be due to synergistic efficacy of buparvaquone and hematinic against tropical theileriosis (Sharma *et al*., 2010). Further, calf recovery from theileriosis was evident with the absence of intra-erythocytic piroplasm and no amplification of 733 bp *TAMS* 1 gene.

This is a report on Theileria induced corneal opacity case of a calf in Jaipur region of Rajasthan (India). Keeping this case in view, each and every suspected case of Theileria should be examined in near future by using classical, serological and molecular tools of investigation, which in turn help in formulating a comprehensive control and preventive strategy.

Conflict of interest: Authors have no conflict of interest in this study.

Author's contribution: KS, BR, SK**,** SB, NSK, MJ**,** PP**,** RV: All authors helped for data collection, analysis and interpretation of results, and manuscript preparation.

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REFERENCES

Boulter N and Hall R, 1999. Immunity and vaccine development in the bovine theilerioses. Adv Parasitol, 44: 41-97, doi: 10.1016/s0065-308x(08)60230-4

d'Oliveira C, van der Weide M, Habela MA, Jacquiet P and Jongejan F, 1995. Detection of *Theileria annulate* in blood samples of carrier cattle by PCR. J Clin Microbiol, 33(10): 2665-2669, doi: 10.1128/jcm.33.10.2665-2669.1995

Gubbels MJ, Katzer F and Hide G, 2000. Generation of a mosaic pattern of diversity in the major merozoite-piroplasm surface antigen of *Theileria annulata*. Mol Biochem Parasitol, 110(1): 23-32, doi: 10.1016/s0166-6851(00)00253-x

Gupta SK, Yadav A, Raina AK and Singh R, 2004. Theileriosis in a seven-day old bovine calf- A case report. Indian J Vet Med, 24: 55

Hudson AT, Randall AW, Fry M, Ginger CD, Hill B *et al*., 1985. Novel anti-malarial hydroxynaphthoquinones with potent broad-spectrum anti protozoal activity. Parasitology, 90(Pt 1): 45-55, doi: 10.1017/S003118200004 9003

Irvin AD and Mwamachi DM, 1983. Clinical and diagnostic features of East Coast fever (*Theileria parva*) infection of cattle. Vet Rec, 113(9): 192-198, doi: 10.1136/vr.113.9.192

Jabbar SA, Elizabeth JG, Diaeldin AS and Ulrike S, 2008. Innate immunity to tropical theileriosis. Innate Immun, 14(1): 5-12, doi: 10.1177/1753425907087258

Joshi V, Alam S, Dimri U, Bhanuprakash AG, Gopalakrishnan A *et al*., 2017. A rare case of *Theileria annulata* induced corneal opacity in a calf. J Parasit Dis, 41(2): 442-445, doi: 10.1007/s12639-016-0824-z

Khan IA, Khan A, Hussain A, Riaz A and Aziz A, 2011. Hematobiochemical alterations in cross bred cattle affected with bovine theileriosis in semi arid zone. Pak Vet J, 31(2): 137-140

Mans BJ, Pienaar R and Latif AA, 2015. A review of Theileria diagnostics and epidemiology. Int J Parasitol Parasites Wildl, 4(1): 104-118, doi: 10.1016/j.ijppaw.2014.12.006

McColm AA and McHardy N, 1984. Evaluation of a range of antimicrobial agents against the parasitic protozoa, *Plasmodium falciparum*, *Babesia rodhaini* and *Theileria parva in vitro*. Ann Trop Med Parasitol, 78(4): 345-354, doi: 10.1080/00034983.1984.11811831

Naik G, Ananda J and Kavitha RB, 2010. Theileriosis in calves and its successful treatment. Vet World, 3(4): 191

Rajendran C, Ray DD and Bansal GC, 2008. Expression of gene encoding immunodominant merozoite surface protein of *Theileria annulata* in *Escherichia coli*. Ind J Biotecnol, 7(2): 200-203

Saini K, Rathore B, Sumbria D, Monika, Varshney R *et al*., 2018. First case report and triumphant treatment of theileriosis in 19 days old crossbred cattle calf in the Jaipur. J Entomol Zool Stud, 6(6): 1059-1061

Sharma S, Gosal NS, Kumar V and Gajebasia AS, 2010. Synergistic efficacy of antitheilerial and haematinic agents in treatment of tropical theileriosis in crossbred cattle. Intas Polivet, 11(1): 23-25

Singh A, Singh J, Grewal AS and Brar RS, 2001. Study on some blood parameters of crossbred calves with experimental *Theileria annulata* infections. Vet Res Commun, 25(4): 289-300, doi: 10.1023/a:1010678625336

Singh SK, Sudan V, Sachan P and Srivastava A, 2015. Salvage of Theileria infected calves with clinical manifestation of exophthalmia. J Parasit Dis, 39(3): 448-451, doi: 10.1007/s12639-013-0364-8

Soulsby EJL, 1982. Helminths, Arthropod and Protozoa of Domesticated Animals, 7th edn. Baillere Tindall, London, PP 809, doi: 10.1016/0035-9203(84)90110-X

Taylor MA, Coop RL and Wall RL, 2007. Veterinary Parasitology, 3rd edn. Blackwell, Oxford

Tuli A, Singla LD, Sharma A, Bal MS, Filia G *et al*., 2015. Molecular epidemiology, risk factors and hematochemical alterations induced by *Theileria annulata* in bovines of Punjab (India). Acta Parasitol, 60(3): 378-390, doi: 10.1515/ap-2015-0053

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