

Atypical theileriosis with cutaneous involvement in a cow: a case report

This paper (No. 14112019-00150-EN) has been peer-reviewed, accepted, edited, and corrected by authors. It has not yet been formatted for printing. It will be published in December 2019 in issue 38 (3) of the *Scientific and Technical Review*.

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Summary

Bovine tropical theileriosis caused by *Theileria annulata* is an overwhelming haemoprotozoan tick-borne disease in taurine and cross-bred cattle in Punjab. However, there seems to be no report from India of cutaneous nodules associated with the disease. This report describes a five-year-old cross-bred cow presented to a university clinic with a history of fever, inappetence and malaise for the past six to seven days. Clinical examination revealed normal vital parameters, pale mucous membranes, mild enlargement of the prescapular lymph nodes and multiple subcutaneous nodular masses (2–4 cm) on the neck and abdomen. Haematology revealed mild anaemia and leucopenia with 48% neutrophils, 48% lymphocytes and 4% eosinophils. Romanowsky stained smears of fine needle aspiration biopsy samples from swollen

lymph nodes and subcutaneous masses showed an increased number of lymphoid cells, suggesting cutaneous lymphomatosis. However, a critical examination of the smears from subcutaneous nodules showed a large number of Koch's blue bodies in macrophages and lymphoblasts, and several piroplasms were also noticed within the red blood cells in lymph node smears. A peripheral blood smear revealed mild to moderate parasitaemia. Extracted DNA from the parasitologically positive blood sample was subjected to nested polymerase chain reaction (nPCR) using *T. annulata* species-specific primers encoding the 30-kiloDalton major sporozoite surface antigen. The desired 572-base pair amplified product of the nPCR was comparable to the positive control. This seems to be a rare case of *T. annulata* in an adult cross-bred cow, showing cutaneous nodular involvement.

Keywords

Cow – Cutaneous – Nested PCR – *Theileria annulata* – Theileriosis.

Introduction

Bovine tropical theileriosis poses major problems for the health and management of cattle and causes huge economic losses in terms of productivity and health in India. It is caused by the tick-borne obligate intracellular protozoan parasite *Theileria annulata* that infects both wild and domestic bovidae throughout the world and is transmitted transstadially by *Hyalomma anatolicum*, a three-host tick, which acts as a biological vector (1). The parasite causes infection of leucocytes by sporozoites, which is followed by maturation of schizonts into merozoites, and subsequent infection of red blood cells (RBCs) with formation of piroplasms. The disease is of great significance because cows that have recovered from acute or primary theileriosis remain carriers for a long period, possibly for their lifetime, acting as reservoirs of infection for ticks (2).

The disease is common in Punjab and is characterised by fever, anaemia and swollen superficial lymph nodes, particularly the prescapular lymph nodes (3, 4). The severity of infection depends on the

susceptibility of the host, the quantum of infection delivered by vector ticks and parasite virulence (5, 6). The chief pathological features of acute lethal tropical theileriosis are associated with dysfunction of the lymphoid and reticulo-endothelial systems, and haematological changes. On farms in a stable endemic state, the clinical signs are usually unnoticed in adults, but 10–20% mortality may occur in calves. In the acute lethal disease, cattle show pronounced clinical signs within five to seven days of infection, and at this stage schizonts become detectable in the lymph node(s) draining the site of infection (7).

This communication reports a rare case of non-fatal theileriosis with cutaneous involvement in an adult cow, suggesting metastasis and development of schizonts in the cutaneous lymphoproliferative nodules, leading to moderate parasitaemia and clinical disease under stress.

Case presentation

A five-year-old cross-bred cow was presented with a history of fever (40.5–41.2°C), inappetence and malaise for the past six to seven days without any evidence of tick infestation. The cow showed a gradual reduction in milk yield. Clinical examination revealed normal body temperature (38.7°C), heart rate (68 beats per minute [bpm]) and respiration rate (24/min), pale mucous membranes and mild enlargement of prescapular lymph nodes. On further examination, nine easily movable subcutaneous nodular masses varying in size from 2 to 4 cm in diameter were palpable on the neck and abdomen (Fig. 1).



Fig. 1

Multiple cutaneous and movable nodular foci at the right paralumbar fossa and on the neck

Materials and methods

A blood sample (2 ml) collected aseptically from the jugular vein was transferred to ethylenediaminetetraacetic acid (EDTA) vials for the analysis of blood cellular components. The blood samples were analysed within 2 h of blood collection for haematology. The blood smears were air dried, stained with Leishman stain, examined with immersion oil under a light microscope and evaluated for the presence of haemoprotozoa.

In addition, fine needle aspiration biopsy (FNAB) samples were taken from the swollen lymph nodes and subcutaneous nodular masses for cytological examination. The DNA was extracted from peripheral blood and subjected to nested polymerase chain reaction (nPCR) using *T. annulata* species-specific primers targeting the 30-kiloDalton (kDa) major merozoite surface antigen (*Tams 1*) (8).

Results

Haematology revealed 6.9 g/l haemoglobin and a total leucocyte count (TLC) of 4,200 per mm³ (48% neutrophils, 48% lymphocytes and 4% eosinophils). The blood smear on routine examination appeared negative for *Theileria* initially. However, given the presence of the nodular masses, the case was suspected to involve subcutaneous neoplasia and thus Romanowsky stained smears of the FNAB were prepared.

The cytological examination of the smears from swollen lymph nodes and subcutaneous masses showed increased numbers of lymphoid cells suggesting cutaneous lymphomatosis (Fig. 2). However, a detailed examination of the smear revealed pleomorphic lymphoid cells composed of 50% small lymphocytes along with lymphoblasts and a significant number of macrophages, besides a few neutrophils and plasma cells, indicating a lymphoproliferative disorder.

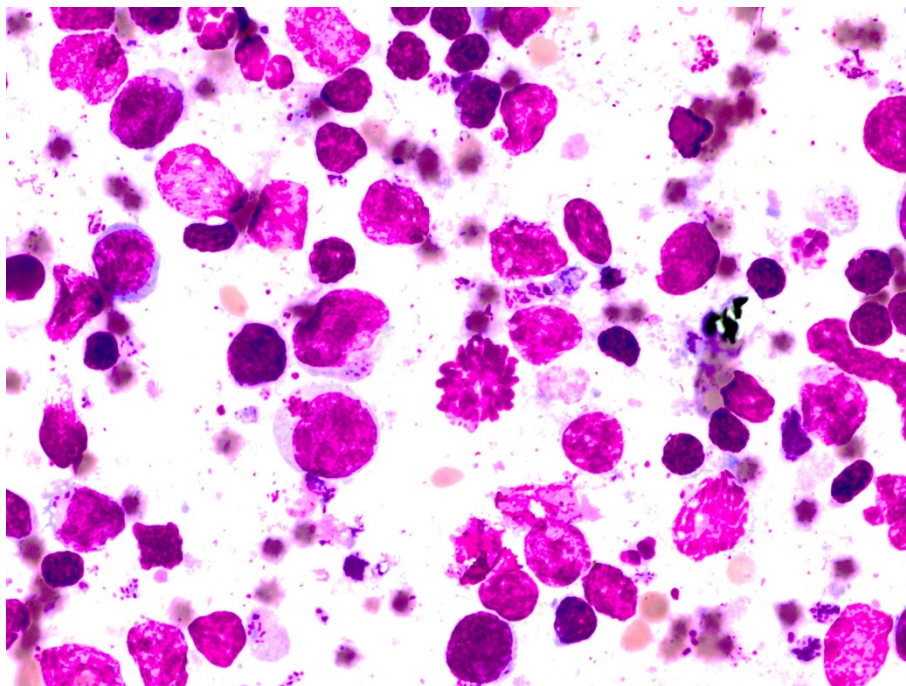


Fig. 2

Fine needle aspiration biopsy sample showing lymphoproliferation and the presence of Koch's blue bodies in lymphoblasts and macrophages as well as outside the cells

Re-staining and further examination of the smears, particularly those from subcutaneous nodules, showed a large number of *Theileria*-like schizonts in various stages of development in the macrophages and lymphoblasts. Some of them were found to be extracellular and even releasing free merozoites (Fig. 3). In addition to schizonts in the macrophages and lymphoid cells, several piroplasms were also noticed within the RBCs of the cytological smears from the cutaneous nodules. For confirmation, more FNABs were taken from different subcutaneous masses and stained with Leishman stain. Again, the smears from different sites revealed numerous schizonts in lymphocytes and macrophages along with a large number of piroplasms within the RBCs from these masses. However, the aspirates from prescapular and prefemoral lymph nodes contained notably fewer parasitic forms than those from subcutaneous nodular masses. As the blood smear was initially negative for *Theileria*, a peripheral blood smear was prepared later from an ear vein, which revealed piroplasms within the RBCs (Fig. 4), indicating moderate parasitaemia.

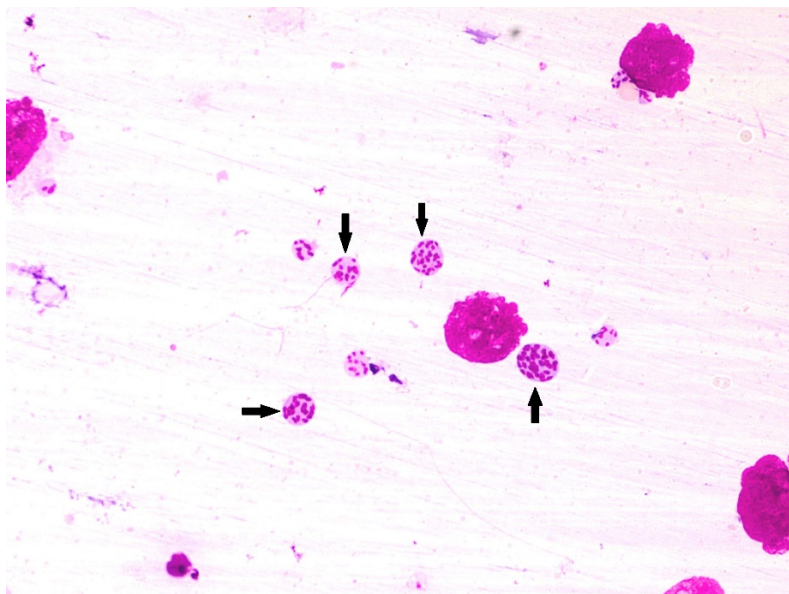


Fig. 3

Several *Theileria* schizonts at different stages of development presenting extra- and intracellularly in a fine needle aspiration biopsy smear. 100× (Leishman stain)

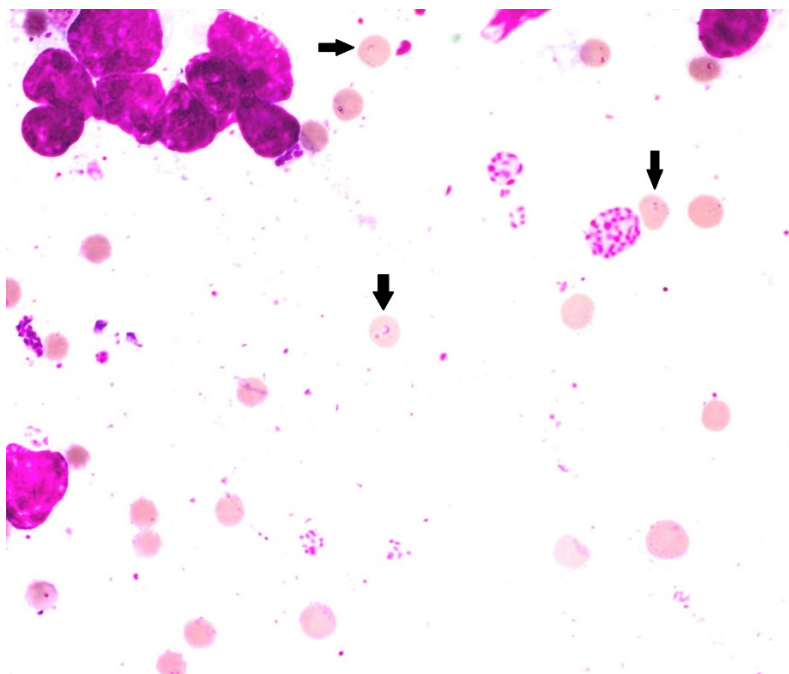


Fig. 4

Presence of piroplasms within the red blood cells (arrows) and free merozoites in a fine needle aspiration biopsy smear. 100× (Leishman stain)

The DNA extracted from the parasitologically positive blood sample was subjected to nPCR using *Theileria annulata* species-specific primers encoding the 30-kDa major sporozoite surface antigen. The desired 572-base pair (bp) amplified product of the nested PCR was comparable to the positive control (Fig. 5).



Fig. 5

Agarose gel electrophoresis showing amplified 572-bp fragment of *Theileria annulata* DNA produced by nested polymerase chain reaction

bp: Base pair

L: 100 bp plus DNA ladder

P: Positive control

N: Non-template control

S: Sample positive for *Theileria annulata* DNA

Based on the clinical signs, laboratory examination and molecular confirmation, the condition was diagnosed to be tropical theileriosis with cutaneous involvement. The cow was treated with a single deep intramuscular injection of buparvaquone at 2.5 mg/kg (ButalexTM, MSD Animal Health, Ahmedabad, India), followed by three intravenous injections of oxytetracycline hydrochloride (Steclin, Zydus Animal Health, Haridwar, Uttarakhand, India) at 10 mg/kg once daily. Supportive treatment comprised BelamylTM (B complex liver extract with vitamin B₁₂ injection, Zydus, Ahmedabad, India) given at 10 ml intramuscularly for five days. After 15 days, the owner reported gradual improvement in the condition of the animal, with regression of the cutaneous nodules, and the milk yield had returned almost to the normal level. The cow was kept on supportive treatment for one more week.

Discussion

This case may be the first in India in which *Theileria* schizonts were found in cutaneous nodules in an adult cow. Previously, urticarial lesions due to theileriosis have been reported in an indigenous cow, which is highly suggestive of the allergic consequence of acute parasitaemia, which may have been responsible for intense pruritus in that animal (9). The metastasis of schizont-infected cells from prescapular lymph nodes to non-lymphoid organs, including the skin, of adult cattle is similar to findings in six calves (10). However, the cells parasitised by *Theileria* in those calves spread rapidly to the liver, kidneys, lungs, abomasum, adrenal glands and pituitary gland but not the skin. It is possible that the tropical theileriosis in the case described herein was caused by parasites living in and disseminated by cytokine secreting, proliferating mononuclear phagocytes (10), and therefore this cannot be considered to be a lymphoproliferative disorder resulting from the uncontrolled multiplication and metastasis of lymphoid cells infected with *T. annulata* schizonts. The other possible mechanism is that the organisms were harboured in the subcutaneous nodules and, with the stress of the onset of winter, the disease flared up, resulting in peripheral blood parasitaemia. Cutaneous lesions have been reported in experimentally infected calves after attachment of *Rhipicephalus appendiculatus* ticks infected with *T. parva*: skin lesions were recorded

after 120 h of infestation (11). However, the nodules in those calves were haemorrhagic nodules distributed throughout the skeletal muscles and causing severe myonecrosis, but not involving the subcutaneous tissues. There have been reports showing natural infection by *T. annulata* in calves with widespread skin nodules (10, 12) but not in an adult animal. Two clinical cases of tropical theileriosis were reported in cattle with the uncommon clinical sign of skin nodules, with total resolution of skin lesions in one animal and death of the other post treatment (13).

The reduced haemoglobin concentration and TLC in the case reported herein could be attributed to the toxic effects of metabolites of *T. annulata* and tumour necrosis factor- α (TNF- α) on erythropoiesis (14). A decrease in TLC has also been reported previously (15). This decrease has been related to destruction of leucocytes in lymphoid organs and infiltration of these cells into other organs (16). However, in another study, a decrease in total erythrocyte count and haemoglobin but an increase in TLC was reported (13).

In this case, nPCR using *T. annulata* species-specific primers encoding the 30-kDa major sporozoite surface antigen confirmed the infection. The prevalence of *T. annulata* infection in the tick vector *Hyalomma anatolicum* collected from healthy animals was reported as 8.3, 20.0 and 60.0% by methyl green–pyronin (MGP) staining, primary PCR and nPCR, respectively (17). In the case of *Theileria*, most assays detect genomic DNA and sensitivity is influenced by DNA extraction efficiency (i.e. yield), gene copy number in the genome, number of parasites (genomic copies) per cell sampled and the level of parasitaemia. During clinical reactions, piroplasm parasitaemia may be as high as 1–45% depending on the parasite species (18), but this generally drops well below 1% in the asymptomatic carrier state (19).

Successful recovery from cutaneous theileriosis in an adult cow has been reported following treatment with buparvaquone and oxytetracycline, used with deltamethrin because ticks were present (13).

Dermatological signs in bovine theileriosis are considered rare. Scattered clinical cases have been reported in several enzootic regions of the world. The presence of skin nodules may indicate a poor prognosis (12, 13). This report seems to be unique, describing the first case of non-fatal theileriosis with cutaneous involvement in an adult cross-bred cow with no recent history of ticks, where the diagnosis was confirmed by cytological and molecular examination, and the animal showed recovery following treatment.

Acknowledgements

The authors gratefully acknowledge Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India for generously providing all the required funds and facilities to carry out this study.

References

1. Salih D.A., El Hussein A.M. & Singla L.D. (2015). – Diagnostic approaches for tick-borne haemoparasitic diseases in livestock. *J. Vet. Med. Anim. Hlth*, **7** (2), 45–56. <https://doi.org/10.5897/JVMAH2014.0345>.
2. Bishop R., Musoke A., Morzaria S., Gardner M. & Nene V. (2004). – *Theileria*: intracellular protozoan parasites of wild and domestic ruminants transmitted by ixodid ticks. *Parasitology*, **129** (S1), S271–S283. <https://doi.org/10.1017/S0031182003004748>.
3. Aulakh G.S. & Singla L.D. (2006). – Clinico-haematobiochemical observations on bovines naturally infected with *Theileria annulata*. *J. Vet. Parasitol.*, **20** (1), 49–52. Available at: www.indianjournals.com/ijor.aspx?target=ijor:jvp&volume=20&issue=1&article=011 (accessed on 4 April 2019).
4. Kumar V., Kaur P., Wadhawan V.M., Pal H., Sharma H. & Kumar P. (2015). – Theileriosis in cattle: prevalence and seasonal incidence in Jalandhar district of Punjab (India). *Int. J. Recent Sci. Res.*, **6** (3), 2998–2999. Available at: www.recentscientific.com/theileriosis-cattle-prevalence-and-seasonal-incidence-jalandhar-district-punjab-india (accessed on 4 April 2019).

5. Mahajan V., Gupta M.P. [...] & Sandhu K.S. (2013). – Outbreaks of theileriosis in cattle in Punjab. *Indian Vet. J.*, **90** (1), 77–78. Available at: www.researchgate.net/publication/261158159_Outbreaks_of_theileriosis_in_cattle_in_Punjab (accessed on 4 April 2019).

6. Vasudev A., Sharma A., Kaur P., Bal M.S. & Singla L.D. (2014). – Application of multiplex PCR for the simultaneous detection of natural infection of theileriosis, babesiosis and trypanosomosis in cattle. *J. Vet. Parasitol.*, **28** (2), 112–116. Available at: www.researchgate.net/publication/294579471_Application_of_Multiplex_PCR_for_the_simultaneous_detection_of_natural_infection_of_Theileriosis_Babesiosis_and_Trypanosomosis_in_cattle (accessed on 4 April 2019).

7. Mans B.J., Pienaar R. & Latif A.A. (2015). – A review of *Theileria* diagnostics and epidemiology. *Int. J. Parasitol. Parasites Wildl.*, **4** (1), 104–118. <https://doi.org/10.1016/j.ijppaw.2014.12.006>.

8. d'Oliveira C., van der Weide M., Jacquet P. & Jongejan F. (1997). – Detection of *Theileria annulata* by the PCR in ticks (Acari: Ixodidae) collected from cattle in Mauritania. *Experim. Appl. Acarol.*, **21** (5), 279–291. <https://doi.org/10.1023/A:1018455223462>.

9. Sudhan N.A., Prasad K.D., Sinha A.K., Azmi S. & Sinha K.P. (1992). – A case of cutaneous theileriosis in an indigenous cow. *Indian Vet. J.*, **69** (1), 59–60.

10. Forsyth L.M.G., Minns F.C., Kirvar E., Adamson R.E., Hall F.R., McOrist S., Brown C.G.D. & Preston P.M. (1999). – Tissue damage in cattle infected with *Theileria annulata* accompanied by metastasis of cytokine-producing, schizont-infected mononuclear phagocytes. *J. Comp. Pathol.*, **120** (1), 39–57. <https://doi.org/10.1053/jcpa.1998.0256>.

11. Kimeto B.A. (1978). – Histopathologic and electron microscopic studies of cutaneous lesions in calves with experimentally induced East Coast fever (theileriosis) [abstract]. *Am. J. Vet. Res.*,

39 (7), 1117–1122. Available at: <http://europepmc.org/abstract/med/98077> (accessed on 21 April 2019).

12. Manickam R., Dhar S., Singh R.P. & Kharole M.U. (1984). – Histopathology of cutaneous lesions in *Theileria annulata* infection of calves [abstract]. *Indian Vet. J.*, **61** (1), 13–15. Available at: www.cabdirect.org/cabdirect/abstract/19842247774 (accessed on 21 April 2019).

13. Gharbi M., Souidi K., Boussaadoun M.-A., Rejeb A., Jabloun S., Gnaoui A. & Darghouth M.-A. (2017). – Dermatological signs in bovine tropical theileriosis (*Theileria annulata* infection), a review. *Rev. Sci. Tech. Off. Int. Epiz.*, **36** (3), 807–816. <https://doi.org/10.20506/rst.36.3.2716>.

14. Boulter N. & Hall R. (1999). – Immunity and vaccine development in the bovine theilerioses. *Adv. Parasitol.*, **44**, 41–97. [https://doi.org/10.1016/S0065-308X\(08\)60230-4](https://doi.org/10.1016/S0065-308X(08)60230-4).

15. Goddeeris B.M., Katende J.M., Irvin A.D. & Chumo R.S.C. (1982). – Indirect fluorescent antibody test for experimental and epizootiological studies on East Coast fever (*Theileria parva* infection in cattle). Evaluation of a cell culture schizont antigen fixed and stored in suspension. *Res. Vet. Sci.*, **33** (3), 360–365. [https://doi.org/10.1016/S0034-5288\(18\)32316-6](https://doi.org/10.1016/S0034-5288(18)32316-6).

16. Clark I.A., Hunt N.H. & Cowden W.B. (1986). – Oxygen-derived free radicals in the pathogenesis of parasitic disease. *Adv. Parasitol.*, **25**, 1–44. [https://doi.org/10.1016/S0065-308X\(08\)60341-3](https://doi.org/10.1016/S0065-308X(08)60341-3).

17. Tiwari A., Singh N.K., Singh H., Jyoti, Bhat S.A. & Rath S.S. (2015). – Prevalence of *Theileria annulata* infection in *Hyalomma anatolicum anatolicum* collected from crossbred cattle of Ludhiana, Punjab. *J. Parasit. Dis.*, **39** (1), 57–61. <https://doi.org/10.1007/s12639-013-0279-4>.

18. Schetters Th.P.M., Arts G., Niessen R. & Schaap D. (2010). – Development of a new score to estimate clinical East Coast fever in

experimentally infected cattle. *Vet. Parasitol.*, **167** (2–4), 255–259. <https://doi.org/10.1016/j.vetpar.2009.09.027>.

19. Ueti M.W., Mealey R.H. [...] & Knowles D.P. (2012). – Re-emergence of the apicomplexan *Theileria equi* in the United States: elimination of persistent infection and transmission risk. *PLoS ONE*, **7** (9), e44713. <https://doi.org/10.1371/journal.pone.0044713>.
