A rare case of *Theileria annulata* induced corneal opacity in a calf

Vivek Joshi¹ · S. Alam¹ · U. Dimri¹ · A. G. Bhanuprakash² · A. Gopalakrishnan¹ · Y. Ajith²

Received: 21 July 2016 / Accepted: 8 August 2016 / Published online: 13 August 2016
© Indian Society for Parasitology 2016

Abstract A 10 months old male cross-bred calf was presented in OPD Medicine, Referral Veterinary Polyclinic, ICAR-Indian Veterinary Research Institute, Izatnagar with the history of anorexia, impaired vision, heavy tick infestation and diarrhoea for the last 1 week. The clinical examination revealed enlarged pre-scapular lymph nodes, prominent cloudiness in eyes and exophthalmos. The presence of Koch blue bodies in biopsied lymph nodes and a PCR assay confirmed it to be bovine tropical theileriosis associated with unique corneal opacity. Treatment with buparvaquone at a dose of 2.5 mg/kg i.m. two doses 48 h apart was successful and typical ocular manifestations completely subsided after 2 weeks of therapy.

Keywords Buparvaquone · Exophthalmos · Opacity · Bovine tropical theileriosis

Introduction

In India, bovine tropical theileriosis (BTT) is considered to be one of the life-threatening diseases common in dairy animals (Tuli et al. 2015). It is caused by an apicomplexan parasite of red cells, *Theileria annulata* which is transmitted by the bite of a tick, *Hyalomma anatolicum* (Taylor et al. 2007). The initial chief symptoms include enlargement of regional superficial lymph nodes, high fever

(40.5–41.5 °C), laboured breathing, lacrimation followed by anemia and profound leucopenia (Khan et al. 2011). A marked anemia caused by *T. annulata* occurs due to erythrophagocytosis (Devi et al. 2011; Aulakh and Singla 2006; Muraleedharan et al. 2005) and it delays the recovery and survival of infected cattle even when treated by highly efficacious antitheilerial drugs (Dhar et al. 1986). Immunity related genes are amplified by means of polymerase chain reaction (PCR) and this PCR based assay is useful to make a molecular diagnosis of *T. annulata* in cattle even when a small amount of parasite DNA is present in blood (Dewangan et al. 2015).

The clinical cases of *T. annulata* induced corneal opacity are rarely seen and hardly recorded in regular practice (Singh et al. 2015). This study reports a unique case of theileriosis associated corneal opacity in a cross-bred calf which was confirmed by parasitological as well as by molecular means (PCR).

History and clinical observations

A 10 month old cross-breed calf was brought to the Referral Veterinary Polyclinic, ICAR-Indian Veterinary Research Institute, Izatnagar with history of anorexia, lacrimation, impaired vision, sternal recumbency, heavy tick infestation and weight loss for the last 1 week. The clinical examination showed the presence of enlarged pre-scapular lymph nodes (Fig. 1), tachycardia (77 bpm) and a high fever (104.2 °F). The calf was subjected to examination of mucosal membranes (conjunctival, nasal and oral) for the presence of anemia. Thin blood smear was prepared from the peripheral blood sample collected from the ear tip of calf. The smears were air-dried, fixed with methanol, stained with 10 % Giemsa stain and examined

✉ Vivek Joshi
joshignet@gmail.com

¹ Clinical Medicine Laboratory, ICAR-Indian Veterinary Research Institute, Izatnagar, UP 243122, India

² Division of Medicine, ICAR-Indian Veterinary Research Institute, Izatnagar, UP 243122, India

Fig. 1 Enlarged prescapular lymph node

Fig. 2 Corneal opacity and lacrimation

under oil immersion objective of microscope (Soulsby 1982) which revealed signet ring-shaped piroplasms of *T. annulata* in erythrocytes (Fig. 3). Blood sample was also collected for hemato-biochemical analysis. Hematology showed 8 g haemoglobin and 24 % PCV indicative of developing anemia. The ophthalmic examination revealed profuse bilateral lacrimation, bulging eyes, supraorbital oedema, cloudy eyes, corneal opacity and impaired vision (Fig. 2).

Lymph node biopsy smear and ticks from the thigh and neck were collected, and examined in Division of Parasitology, ICAR-Indian Veterinary Research Institute, as per the standard recommended procedures. Koch blue bodies (KBB) (Fig. 4) observed in lymphoblasts confirmed it to be

Fig. 3 Signet ring piroplasms in RBCs

Fig. 4 Koch blue bodies in lymphoblast

a case of tropical bovine theileriosis with uniqueness regarding presence of corneal opacity. Further, molecular confirmation of *T. annulata* infection was done by a PCR based assay (D'oliveira et al. 1995) in Division of Animal Genetics, ICAR-Indian Veterinary Research Institute, where targeting the SSU rRNA gene, amplification of a 372-bp fragment was done (Fig. 5) using 989/1347 forward and reverse primers, respectively (Table 1).

Treatment

The calf was treated with two doses of buparvaquone (Butalex, Zydus Animal Health Ltd.) at the dose of 2.5 mg/kg i.m. at 48 h interval (Qayyum et al. 2010; Muhammad 1999). The adjunct therapy included meloxicam (Melonex, Intas Pharmaceuticals Ltd.) at the dose of 0.5 mg/kg i.m.,

Fig. 5 PCR amplification of a 372 bp DNA fragment of *T. annulata*. Lane M 100 bp DNA marker, Lane 1 Clinical sample from calf, Lane 2 Positive control

Table 1 *T. annulata* specific primers for PCR based molecular detection

Primer set	Primer sequence (5′–3′)	Primer specificity
Forward primer	AGTTTCTGACCTATCAG	<i>Theileria</i> species
Reverse primer	TGCACAGACCCAGAGG	<i>T. annulata</i>

chlorpheniramine maleate (Cadistin, Zydus Animal Health Ltd.) 0.5 mg/kg i.m., multivitamins (Vitakey, Zydus Animal Health Ltd.) 2 ml i.m for 5 days and five injections of a hematinic (Feritas, Intas Pharmaceuticals Ltd.) at the rate of 100 mg i.m. on 0, 3rd, 6th, 9th and 12th day of therapy. Also, herbal anti-diarrhoeal powder (Neblon, Natural Remedies Pvt. Ltd.) at the rate of 50 g orally twice daily with rice water and second day, one dose of long acting oxytetracycline (Oxytetracycline-LA, Zydus Animal Health Ltd.) at the rate of 20 mg/kg was given intramuscularly. Antipyretic, antihistaminic and anti-diarrhoeal preparations were employed depending upon the disappearance of clinical signs. The application of topical deltamethrin spray (Butox, MSD Animal Health) at a dilution of 2 ml/l of water was also recommended. The improvement was observed 2 days after treatment and calf recovered completely 2 weeks post-therapy.

Discussion

Buparvaquone is very effective and highly specific for the treatment of clinical cases of bovine tropical theileriosis in early as well as late stages of infection (McHardy and Morgan 1985) and it acts by hampering the mitochondrial electron transport chain of *Theileria* parasite (McColm and McHardy 1984; Hudson et al. 1985). The combined administration of buparvaquone and hematinic shows synergistic efficacy against tropical theileriosis (Sharma et al. 2010). In cattle, corneal opacity associated with *T. annulata* is a rare ophthalmopathy. Corneal opacity in theileriosis is attributed to the invasion of cornea, lens and iris by leucocytes (Irvin and Mwamachi 1983). The endogenous pyrogen released in the blood due to cellular lysis leads to fever and consequently, inappetence (Glass et al. 2003). Macroschizonts multiplication occurs inside the lymphocyte causing inflammatory reaction in early stages of theileriosis and enlargement of superficial lymph nodes is the result of lymphoid hyperplasia (Jabbar et al. 2008). Anemia occurs due to erythrophagocytosis and subsequent removal by reticuloendothelial system (Singh et al. 2001). PCR along with conventional Giemsa staining are very useful techniques for diagnosis of *T. annulata* in clinical infection. The sensitivity of PCR assay is high since it detects *Theileria* parasite even when the parasitemia rate is low (Parthiban et al. 2010).

Acknowledgments The authors are thankful to Director, ICAR-Indian Veterinary Research Institute for providing necessary research facilities to carry out this work. The authors also express sincere thanks to Dr. S. Gupta, Division of Parasitology and Dr. A. Kumar, Division of Animal Genetics, ICAR-Indian Veterinary Research Institute for the help extended during parasitological and molecular detection of *T. annulata*, respectively.

Authors' contributions The study reports one of the rare clinical cases being mutually diagnosed and treated by Vivek Joshi and S. Alam. U. Dimri provided the guidelines during the work. A. G. Bhanuprakash, A. Gopalakrishnan, and Y. Ajith helped in correction of manuscript.

Compliance with ethical standards

Conflicts of interest None of the authors has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

References

- Aulakh GS, Singla LD (2006) Clinico-haematobiochemical observations on bovines naturally infected with *Theileria annulata*. J Vet Parasitol 20:49–52
- D'oliveira C, Weide MVD, Miguel A, Jacquet HP, Jongejan F (1995) Detection of *Theileria annulata* in blood samples of carrier cattle by PCR. J Clin Microbiol 33:2665–2669

- Devi CS, Rani NL, Rayulu VC (2011) Concurrent infection of theileriosis and ehrlichiosis in a buffalo. *J Vet Parasitol* 25:84–85
- Dewangan P, Panigrahi M, Kumar A, Saravanan BC, Ghosh S, Asaf VM, Bhushan B (2015) The mRNA expression of immune-related genes in crossbred and Tharparkar cattle in response to in vitro infection with *Theileria annulata*. *Mol Biol Rep* 42:1247–1255
- Dhar S, Malhotra DV, Bhushan C, Gautam OP (1986) Chemotherapy of *Theileria annulata* infection with buparvaquone. *Vet Rec* 119:635–636
- Glass EJ, Craigmyle SC, Springbett A, Preston PM, Kirvar E, Wilkie GM, Eckersall PD, Hall FR, Brown CGD (2003) The protozoan parasite *Theileria annulata* induces a distinct acute phase protein response in the cattle that is associated with pathology. *Int J Parasitol* 33:1409–1418
- Hudson AT, Randall AW, Fry M, Ginger CD, Hill B, Latter VS, McHardy N, Williams RB (1985) Novel anti-malarial hydroxynaphthoquinones with potent broad-spectrum anti-protozoal activity. *Parasitology* 90:45–55
- Irvin AD, Mwamachi DM (1983) Clinical and diagnostic features of East Coast fever (*Theileria parva*) infection of cattle. *Vet Rec* 113:192–198
- Jabbar SA, Elizabeth JG, Diaeldin AS, Ulrike S (2008) Innate immunity to tropical theileriosis. <http://ini.sagepub.com>
- Khan IA, Khan A, Hussain A, Riaz A, Aziz A (2011) Hemato-biochemical alterations in cross bred cattle affected with bovine theileriosis in semi arid zone. *Pak Vet J* 31:137–140
- McColm AA, McHardy N (1984) Evaluation of a range of antimicrobial agents against the parasitic protozoa, *Plasmodium falciparum*, *Babesia rodhaini* and *Theileria parva* in vitro. *Trop Med Parasitol* 78:345
- McHardy N, Morgan DW (1985) Treatment of *Theileria annulata* infection in calves with parvaquone. *Res Vet Sci* 39:1–4
- Muhammad G (1999) Clinico-epidemiological and therapeutic aspects of bovine theileriosis. *Bull Calf Appeal Female* 36:32
- Muraleedharan K, Ziauddin KS, Hussain PM, Puttabyattappa R, Seshadri SJ (2005) Haematological observations on *Theileria annulata* infection in cattle and buffaloes. *J Vet Parasitol* 19:71–72
- Parthiban M, Saranya R, Magesh M, Raman M (2010) Detection of *Theileria* parasite in cattle of Tamilnadu using nested PCR. *Tamilnadu J Vet Anim Sci* 6:162–165
- Qayyum M, Farooq U, Samad HA, Chauhdry HR (2010) Prevalence, clinicotherapeutic and prophylactic studies on theileriosis in district Sahiwal (Pakistan). *J Anim Plant Sci* 20:266
- Sharma S, Gosal NS, Kumar V, Gajebasia AS (2010) Synergistic efficacy of antitheilerial and haematonic agents in treatment of tropical theileriosis in crossbred cattle. *Intas Polivet* 11:23–25
- Singh A, Singh J, Grewal AS, Brar RS (2001) Study on some blood parameters of crossbred calves with experimental *Theileria annulata* infections. *Vet Res Commun* 25:289–300
- Singh SK, Sudan V, Sachan P, Srivastava A (2015) Salvage of *Theileria* infected calves with clinical manifestation of exophthalmia. *J Parasit Dis* 39:448–451
- Soulsby EJJ (1982) Helminths, arthropod and protozoa of domesticated animals, 7th edn. Baillere Tindall, London
- Taylor MA, Coop RL, Wall RL (2007) Veterinary parasitology, 3rd edn. Blackwell, Oxford
- Tuli A, Singla LD, Sharma A, Bal MS, Filia G, Kaur P (2015) Molecular epidemiology, risk factors and hematochemical alterations induced by *Theileria annulata* in bovines of Punjab (India). *Acta Parasitol* 60:378–390