Case Report

Concurrent Trypanosomosis, Babesiosis and Toxocarosis in a Dog: a Case Report

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Abstract

Background: Among the blood-borne parasitic infections of dogs, trypanosomosis and babesiosis are highly prevalent in the Indian subcontinent and highly pathogenic.
Methods: A six-month-old dog was presented with complaint of lethargy, anorexia and loss of body weight over the previous 15 days on October 11, 2021. A full clinical and targeted haematologic and parasitological examination was undertaken.
Results: Wet smears were positive for the motile trypanosomes while stained thin blood smears showed large number of extracellular trypanosomes and intra-erythrocytic ring-like inclusion bodies of *Babesia* spp. A high burden of *Toxocara canis* was identified on coprological examination.
Conclusion: This report describes the clinical manifestation of concurrent infection of haemoparasites and gastrointestinal nematodes in Pakistani dogs, and highlights the importance of prompt veterinary intervention.

Keywords: *Trypanosoma*; *Babesia*; *Toxocara canis*; Dog; Pakistan

Introduction

The haemoprotozoan parasites like *Babesia* (*B.*) *canis*, *B. gibsoni* and *Trypanosoma* (*T.*) *evansi* are common causes of life-threatening diseases in wide range of animals including dogs throughout the Indian subcontinent and in other parts of world (1-5). Salivarian trypanosomes are flagellate haemoprotozoa which belong to family Trypanosomatidae and the genus *Trypanosoma* (6), and are biologically transmitted by biting flies (7). *Trypanosoma evansi* is widely recognized as a cause of neuropathy, immune suppression and anemia in camels and horses, referred to as surra (8, 9); but adult dogs are also widely infected in Pakistan (10, 11). The

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clinical signs of trypanosomosis in dogs include fever, anemia, superficial lymph node enlargement, loss of appetite, lethargy and progressive loss of bodily condition, leading to fatal outcomes if untreated (12). *Babesia* spp. are intraerythrocytic parasites, biologically transmitted by *Haemaphysalis* or *Rhipicephalus* spp. ticks and infecting a wide variety of hosts including humans as well as domestic and wild animals (13). Many species of *Babesia* can infect dogs, causing anemia and hemoglobinuria due to haemolysis as the parasite replicates asexually in the red blood cells (14). The parasites complete their life history through sexual reproduction in the tick host mid gut and the formation of sporozoites in the salivary gland (15).

Canine gastrointestinal nematode (GIN) infections, especially *Toxocara* (*T.*) *canis* and *Ancylostoma* spp., are important causes of retarded growth and general malaise (16). Dogs and humans have a close association (17), and their GINs are of great concern due to their serious zoonotic potential (16). *Toxocara canis* is distributed worldwide, causing diarrhea, poor growth and death if present in large number (18). Environmental contamination with faeces containing vast number of eggs which shed during patent infections presents a serious public health risk (19), resulting in human disease referred to as visceral larval migrans. This case report presents the co-infection of *Trypanosoma* spp., and *Babesia* spp. haemoparasites along with the GIN, *Toxocara canis*, and describes the therapeutic response to treatment in a dog.

Case presentation

A six-month-old male pet dog (Pointer crossbred) was brought to the outdoor clinic at the Khan Bahadhar Chaudhry Mushtaq Ahmad (KBCMA) College of Veterinary and Animal Sciences, Narowal, sub-campus of University of Veterinary and Animal Sciences Lahore, Pakistan on October 11, 2021. The owner reported that the dog had been anorexic, dull and depressed for the past 15 days, and that the onset of clinical signs coincided with the dog having consumed a putrified dead lamb carcass. On examination, the dog was thin, weak and did not respond well to its surroundings (Fig. 1). The rectal temperature was normal (38.8 ^{o}C), oral mucous membranes were pale and the conjunctivae were pale and jaundiced (Fig. 2). There were no swollen lymph nodes, but signs of tachypnoea (38 breaths per minute), coughing and a frothy nasal discharge were observed.

With the consent of the owner, a 5ml blood sample was collected from the cephalic vein into an EDTA coated vacutainer to investigate hematologic parameters and detect any haemoparasites. Sample was collected for coprological examination and processed qualitatively by the faecal floatation technique and quantitatively by a McMaster egg counting method using three-chambered slide having threshold of 8 eggs per gram (EPG) (Chalex®, USA). Wet mounts were prepared for motile trypanosomes and the thin blood smears were stained with Field's stain and examined at 1,000x under oil immersion to detect haemoparasites.

The haematologic parameters (Table 1) showed significantly low packed cell volume (PCV) and haemoglobulin (Hb) values indicating severe anemia, and marked eosinophilia, lymphocytosis and neutropenia. The wet mount of blood sample showed the presence of large number of motile extracellular trypanosomes. The Field-stained thin smear confirmed their identity (Fig. 3) and showed the presence of multiple intracellular *Babesia* spp. with ring-like forms in the red blood cells (Fig. 4).

Toxocara canis eggs were identified on coprological examination, being about 75µm x 90 µm in size and having a dark brown colour, a thick pitted shell and sub-globular shape (Fig. 5). An adult worm of *T. canis* was also found in the faeces measuring approximately 18cm in length. The *T. canis* faecal egg count was 2900 EPG, indicating a moderate to high level of infection.

The disease history and clinical signs were consistent with chronic haemoprotozoal infec-

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tion and GIN infection (12); hence the dog was treated with intramuscular injection of diminazene aceturate (Pronil® Injection, Selmore) at a dose rate of 3.5mg/kg body weight on the first day and same dose was repeated after 72 hours. Doxycycline (Vibramycin®, Pfizer) was given orally at a dose rate of 5mg/kg of body weight, along with a liver tonic (1teaspoon per day, Hepa-Merz® Syrup, Brookes) and multivitamins (1 teaspoon per day, ViDayLin-L® Syrup, Abbott) for 5 days. A single treatment of anthelmintic 1 tablet per 10kg body weight (Drontal® Plus, Bayer) was given. The dog did not respond to supportive therapy and drug treatments, and died 20 days after clinical presentation.

Fig. 1. Dog showing emaciation and persistent recumbency

Fig. 2. Dog showing anaemic conjunctiva and an absence of corneal opacity

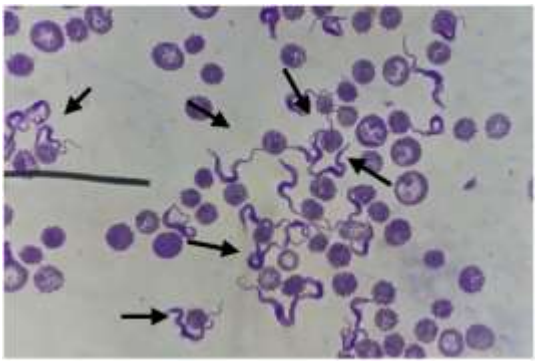

Fig. 3. *Trypanosoma* spp. (arrows) on a Field-stained thin blood smear (1000 x magnification) of an infected dog. The genus identity was confirmed by the morphology of extracellular protozoa with a flagellum, kinetoplast and undulating membrane

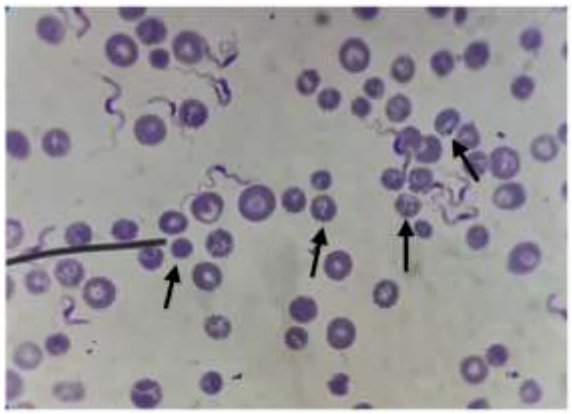

Fig. 4. Erythrocytes showing ring-like forms of *Babesia* spp. (arrows) on a Field-stained thin blood smear (1000 x magnification) of an infected dog

Fig. 5. *Toxocara canis* eggs at 40 x identified on coprological examination of an infected dog

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Table 1. Summary of haematologic parameters of the infected study dog

Hematologic parameters	Observed values	Reference values
Erythrocytes (10^{12}/L)	1.69	5.5-8.5
Hemoglobin (g/dL)	3.70	12-18
Haematocrit (%)	14.0	37-55
Leucocytes (10^9/L)	17.0	6.0-17
Neutrophils (10^9 /L)	1.50	2.9-12
Eosinophils (10^9 /L)	2.0	0.0-1.3
Granulocytes (%)	41.1	60–70
Lymphocytes (%)	49.7	8.0–21
Platelets (10^9 /L)	33.0	200–500

Discussion

Disruption of the erythrocytes by the release of *Babesia* spp. piroplasms and mechanical injury to erythrocytes by circulating trypanosomes would have accounted for the clinical signs that were observed (20, 21). Eosinophilia, and lymphocytosis are typical in case of trypanosomosis (22, 23).

Trypanosomal infections in dogs have been reported in different countries in Asia (10, 21, 24), southern Europe (25), Africa, north America (26) and South America (27). Salivarian trypanosomiasis in various animal hosts caused by *T. evansi*, *T. brucei*, *T. vivax*, *T. congolense* or *T. theileri*, is transmitted by biological vectors such as tsetse fly, and tabanids or *Stomoxys* spp. biting flies, depending upon the *Trypanosoma* spp. and geographical location (28). In Pakistan, the predominant species is *T. evansi*, which is considered to be the result of the bites of the flies of family *Tabanidae* and *Stomoxidae*, and the molecular studies should be performed to confirm the presence of species of this parasite in animals (29, 30). *Babesia canis* and *B. gibsoni* are important causes of disease in dogs with a similar global distribution to *Trypanosoma* spp. but dependent on the distribution of *Dermacentor* spp., *Haemaphysalis* spp. or *Rhipicephalus* tick vectors. The ring-like morphology of the piroplasms identified in this case are consistent with *B. gibsoni*, which is particularly pathogenic and prevalent in the Indian subcontinent, causing mainly chronic disease. The treatment response of these cases to antiprotozoal drugs is generally poor (31). Male dogs are considered more susceptible to *Babesia* infection due to more exposure to tick infestation because of their roaming behaviour in search of mates (32), but the dog in the present case was a pet dog, and the development of infection of *Trypanosoma* and *Babesia* might be due to its being less trypanotolerant and having had less exposure to ticks at a young age (due to provision of more care and hygienic environment as compared to roaming dogs), resulting in less protective immunity. Babesiosis may also be predisposed to immunosuppression caused by *Trypanosoma* spp. (33, 34).

Toxocara canis infection is common in dogs worldwide, and particularly common in the Indian subcontinent (35, 36). Patent infections are usually seen in young puppies, following prenatal placental infection, lactogenic infection, ingestion of eggs or ingestion of a paratenic host. From about 3 months-old, second stage larvae ingested with eggs usually migrate to various tissues, where they become dormant. The high level of infection in a six month-old dog was unusual, and may have been predisposed to by immunosuppression due to trypanosomosis.

Conclusion

The poor response to diminazene aceturate and doxycycline was not unexpected, due to the advanced stage of disease in the dog, and

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highlights the importance of seeking veterinary advice and treatment immediately when signs of ill health are observed.

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Ethical considerations

With the consent of the owner, a 5ml blood sample was collected from the cephalic vein into an EDTA coated vacutainer to investigate hematologic parameters and detect any haemoparasites.

Conflict of interest statement

Authors declare that there is no conflict of interest.

References

1. Aregawi WG, Agga GE, Abdi RD, Büscher P (2019) Systematic review and meta-analysis on the global distribution, host range, and prevalence of *Trypanosoma evansi*. Parasit Vectors. 12(1): 1–25.
2. Ali KM, Hassan EA, Abuowarda MM, Mahmoud MA, Torad FA (2021) Bilateral panophthalmia as a late sequel of leishmaniasis in dogs. Pak Vet J. 41(1): 13–18.
3. Ceylan O, Uslu A, Oztürk O, Sevinc F (2021) Serological investigation of some vector-borne parasitic and rickettsial agents in dogs in the western part of Turkey. Pak Vet J. 41(3): 386–392.
4. Selim A, Abdelhady A, Alahadeb J (2021) Prevalence and first molecular characterization of Ehrlichia canis in Egyptian dogs. Pak Vet J, 41(1): 117–121.
5. Yasein G, Ashraf K, Naveed U, Rashid MI, Shabbir MZ (2022) First genetic evidence of Trypanosoma theileri in indigenous cattle in Southern Punjab Province of Pakistan. Pak Vet J. 42(3): 322–327.
6. Sobhy HM, Barghash SM, Behour TS, Razin EA (2017) Seasonal fluctuation of trypanosomiasis in camels in North-West Egypt and effect of age, sex, location, health status and vector abundance on the prevalence. Beni-Suef Univ J Basic Appl Sci. 6: 64–68.
7. Luciani M, Di Febo T, Orsini M, Krasteva I, Cattaneo A, Podaliri Vulpiani M, Di Pancrazio C, Bachi A, Tittarelli M (2018) *Trypanosoma equiperdum* low molecular weight proteins as candidates for specific serological diagnosis of dourine. Front Vet Sci. 5: 40.
8. Santos FM, Carvalho de Macedo G, Barreto WTG, Oliveira-Santos LGR, Garcia CM, Mourão GM, Porfírio GEO, Marino ED, André MR, Perles L, de Oliveira CE, de Andrade GB, Jansen AM, Herrera HM (2018) Outcomes of *Trypanosoma cruzi* and *Trypanosoma evansi* infections on health of southern coati (*Nasua nasua*), crab-eating fox (*Cerdocyon thous*), and ocelot (*Leopardus pardalis*) in the Brazilian Pantanal. PLoS One. 13: e0201357.
9. Kandeel M, Al-Taher A (2021) Molecular landscapes of deoxyuridine 5'-triphosphatase (dUTPase) as a drug target against camel Trypanosoma evansi. Pak Vet J. 41(2): 235–241.
10. Rashid A, Rasheed K, Hussain A (2008) Trypanosomiasis in dog; a case report. Iran J Arthropod Borne Dis. 2: 48–51.
11. Rashid I, Firyal S, Tariq A, Muhammad G, Saqib M, Zafar MS (2014) Use of Cymelarsan® and manganese chloride for treatment of the canine trypanosomiasis

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(surra): a research report. Adv Anim Vet Sci. 2: 104–105.
12. Da Silva AS, Zanette RA, Wolkmer P, Costa MM, Garcia HA, Lopes ST, Santurio JM, Teixeira MM, Monteiro SG (2009) Diminazene aceturate in the control of *Trypanosoma evansi* infection in cats. Vet Parasitol. 165(1–2): 47–50.
13. Schnittger L, Rodriguez AE, Florin-Christensen M, Morrison DA (2012) *Babesia*: a world emerging. Infect Genet Evol. 12(8): 1788–1809.
14. Baneth G (2018) Antiprotozoal treatment of canine babesiosis. Vet Parasitol. 254: 58–63.
15. Liberska J, Michalik J, Pers-Kamczyc E, Wierzbicka A, Lane RS, Rączka G, Opalińska P, Skorupski M, Dabert M (2021) Prevalence of *Babesia canis* DNA in *Ixodes ricinus* ticks collected in forest and urban ecosystems in west-central Poland. Ticks Tick Borne Dis. 12(5): 101786.
16. Raza A, Rand J, Qamar AG, Jabbar A, Kopp S (2018) Gastrointestinal parasites in shelter dogs: occurrence, pathology, treatment and risk to shelter workers. Animals. 8 (7): 108.
17. Meyer I, Björn F (2014) Dog and owner characteristics affecting the dog–owner relationship. J Vet Behav. 9(4): 143–150.
18. Overgaauw PA, van Knapen F (2013) Veterinary and public health aspects of *Toxocara* spp. Vet Parasitol. 193(4): 398–403.
19. Holland CV (2017) Knowledge gaps in the epidemiology of Toxocara: the enigma remains. Parasitology. 144(1): 1–94.
20. Sivajothi S, Rayulu VC, Reddy BS (2013) Haematological and biochemical changes in experimental *Trypanosoma evansi* infection in rabbits. J Parasit Dis. 39(2): 216–220.
21. Reddy BS, Kumari KN, Sivajothi S, Rayulu VC (2016) Haemato-biochemical and thyroxin status in Trypanosoma evansi infected dogs. J Parasit Dis. 40(2): 491–495.
22. Hare WCD, Soulsby EJL, Abt DA (1970) Bovine trypanosomiasis and lymphocytosis parallel studies. Bibl Haematol. 36: 504–517.
23. de Buhr N, Bonilla MC, Jimenez-Soto M, Von Köckritz-Blickwede M, Dolz G (2018) Extracellular trap formation in response to *Trypanosoma cruzi* infection in granulocytes isolated from dogs and common opossums, natural reservoir hosts. Front Microbiol. 9: 966.
24. Prasad KL, Kondaiah PM, Rayulu VC, Srilatha C (2015) Prevalence of canine trypanosomiasis in certain areas of Andhra Pradesh. J Parasit Dis. 39(2): 238–240.
25. Abbas H, Ullah MR, Rizwan HM, Farooqi SH, Ahmad HM, Ateeq MK, Ahmad M (2021) Canine trypanosomosis: a case report. J Hell Vet Medical Soc. 72(1): 2787–2790.
26. Busselman RE, Meyers AC, Zecca IB, Auckland LD, Castro AH, Dowd RE, Curtis-Robles R, Hodo CL, Saunders AB, Hamer SA (2021) High incidence of *Trypanosoma cruzi* infections in dogs directly detected through longitudinal tracking at 10 multi-dog kennels, Texas, USA. PLoS Negl Trop Dis. 15(11): e0009935.
27. Recalde ODS, Fraenkel S, José Tintel M, Arze VP, Ramírez Centurión N, Rolón M, Vega Gómez C (2021) First report of the presence of *Trypanosoma evansi* in dogs from Paraguay applying molecular techniques. Braz J Vet Med. 43(1): e001920.
28. Calvet F, Medkour H, Mediannikov O, Girardet C, Jacob A, Boni M, Davoust B (2020) An African canine trypanosomosis case import: is there a possibility of creating a secondary focus of *Trypanosoma congolense* infection in France? Pathogens. 9(9): 709.
29. Van Den Abbeele J, Rotureau B (2013) New insights in the interactions between African trypanosomes and tsetse flies. Front Cell Infect Microbiol. 3: 63.

http://jad.tums.ac.ir
Published Online: June 31, 2022

30. Duvallet G, Fontenille D, Vincent R (2017) Entomologie Médicale et Vétérinaire. Vol. 1. IRD and Quae Editions, Marseille, France.
31. Singh A, Singh H, Singh NK, Singh ND, Rath SS (2014) Canine babesiosis in northwestern India: molecular detection and assessment of risk factors. Biomed Res Int. 2014: 741785.
32. Daniel LN, Kujul NB, Kemza SY, Ibukunoluwa MR (2016) Retrospective study of the risk factors associated with canine babesiosis diagnosed at veterinary clinic federal college of animal health and production technology, Vom, north central Nigeria, 1999–2006. Int J Sci Appl Res. 1: 86–93.
33. Okubanjo OO, Adeshina OA, Jatau ID, Natala AJ (2013) Prevalence of *Babesia canis* and *Hepatozoon canis* in Zaria, Nigeria. Sokoto J Vet Sci. 11(2): 15–20.
34. Opara M, Adewumi N, Mohammed BR, Obeta SS, Simon MK, Jegede OC, Agbede RIS (2017) Investigations on the haemoprotozoan parasites of Nigerian local breed of dogs in Gwagwalada Federal Capital Territory (FCT), Nigeria. J Parasitol Res. 10: 1–7.
35. Sharma R, Singh BB, Gill JPS, Jenkins E, Singh B (2017) Canine parasitic zoonoses in India: Status and Issues. Rev Sci Tech Off Int Epiz. 36(3): 817–30.
36. Eslahi AV, Badri M, Khorshidi A, Majidiani H, Hooshmand E, Hosseini H, Taghipour A, Foroutan M, Pestehchian N, Firoozeh F, Riahi SM (2020) Prevalence of *Toxocara* and *Toxascaris* infection among human and animals in Iran with meta-analysis approach. BMC Infect Dis. 20(1): 1–7.

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