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Original Research Article

A CASE STUDY OF CANINE TRYPANOSOMIASIS IN NEPAL

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SUMMARY: A 11year-old mixed breed male dog was presented at a private veterinary clinic in Kathmandu, Nepal with clinical signs of high fever, staggering gait, laboured breathing, and twitching of the eyelids. Physical examination showed elevated body temperature (103.3^{0}F), corneal opacity and pale mucous membranes. The blood report showed low haemoglobin count and packed cell volume indicating anaemia, elevated white blood cell count and reduced platelet count. Renal function was impaired, evident by increased blood urea nitrogen and creatinine levels. The case was confirmed by microscopic morphology of the parasite in a blood smear which showed the presence of numerous trypomastigotes, identified as Trypanosoma along with the blood profile, and clinical signs. Two doses of diminazene aceturate 7 days apart at the dose rate of 5mg per kg bwt. deep IM and supportive therapy were provided to the dog. The dog recovered after a week-long course of treatment.

KEYWORDS: canine trypanosomiasis, diminazene aceturate, Nepal

INTRODUCTION

Trypanosomiasis is an economically important zoonotic disease of vertebrates caused by an extracellular haemoprotozoan parasite, *Trypanosoma spp.* (Bhatia *et al*., 2018; Soulsby, 1982). The disease originated as enzootic affecting only wild animals, including rodents, marsupials, and bats which later spread to domestic animals such as horses, camels, cattle, cats and dogs (Rosypal *et al*., 2007; da Costa *et al*., 2015). Canine trypanosomiasis is mainly caused by two Trypanosoma species, *T.evansi* and *T.cruzi*. Another non-pathogenic specie, *T. rangeli*, has also been reported in canines (Eloy and Lucheis, 2009). *T. evansi* causes a condition called Surra in animals including canines and is known to be transmitted via the salivary route of the vector, Tabanid fly. Although considered non-zoonotic, atypical human cases have been reported from Southeast Asia where it is widely distributed (Desquesnes *et al*., 2013). *T. cruzi*, on the other hand, causes Chagas disease and is considered as a disease of zoonotic importance. It is also called American trypanosomiasis. These protozoans are transmitted by Triatomine bugs (kissing bugs) through their faeces upon biting the human host. Dogs mostly get infected by ingesting carcasses along with the hematophagous fly (Kjos *et al*., 2008).

Canine trypanosomiasis can be presented in acute, sub-acute, and chronic forms. The acute form is the most common form in canine species where death occurs within 2-4 weeks after infection with or without exhibiting clinical signs (Soulsby, 1965). Parasitaemia develops within a few days after the infection in sub-acute form and peaks at 2-3 weeks, when clinical signs are most noticeable (Maxfield and Bermudez, 2020). The clinical signs include pyrexia, anorexia, progressive anaemia, loss of condition, conjunctivitis, corneal opacity, oedema of head and throat, difficulty in swallowing, hoarse voice, and staggering gait (Rjeibi *et al*., 2015; Kl *et al*., 2013). If the animal survives this stage of the disease the disease evolves into a chronic form that lasts throughout the animal's life. Visceral damage, cardiovascular disorders, and neurological disturbances are noticed in the chronic form of canine trypanosomiases (Jaiswal *et al*., 2015). Canine trypanosomiasis is frequently encountered in small animal practice in Nepal. However, to date, there are only limited publications regarding the prevalence and case studies of canine trypanosomiasis in Nepal. This case report presents a simple yet effective method of diagnosis along with a successful treatment strategy.

CASE DESCRIPTION

A 11year-old male mixed German Shepherd dog was presented to a private veterinary clinic in

Kathmandu, Nepal with signs of anorexia, mild generalised tremor, difficulty in breathing, sudden loss of condition and inability to weight bearing on its own. The dog was found to be vaccinated against rabies, distemper, hepatitis, parvovirus, parainfluenza, and leptospirosis as per the prescribed schedule. The dog had a history of frequent ectoparasitic infestation, and a few patchy alopecia due to localized mange were observed. The owner reported the condition worsened within the last two days, during which the dog was off food.

Physical examination of the dog revealed a staggering gait, twitching of eyelids and elevated body temperature (103.3^0F). The dog had laboured breathing with a respiratory rate of 11/min, and a weak pulse of 78/ min. Except for mild alopecia, there was no abnormality on the skin. The dog had prolonged capillary refill time (>2 sec), and pale mucus membranes indicating the anaemic condition. The skin turgor test showed that it was moderately dehydrated (8-10%). Corneal opacity was noticeable in the right eye. Additionally, the visual activity test revealed partial blindness in the dog.

The dog was suspected of having blood parasites or a viral infection and was closely monitored in the following hour. In the meantime, whole blood and serum were sent to the hospital laboratory for Complete Blood Count (CBC) and General Health Plan (GHP). Treatment with anticonvulsive (phenobarbital sodium 15 mg/kg, IV), and antipyretic drug (paracetamol 10 mg/kg PO) were initiated. Ranitidine (50 mg in 2 ml) 0.5 ml SC was injected to reduce stomach acid. Fluid therapy with Ringer's lactate solution (RL), later changed to normal saline (0.9% NaCl) was provided to overcome dehydration. Additionally, 50 ml Dextrose 25% bolus was also administered to reduce the hypoglycaemic condition caused due to starvation.

Blood Examination:

About 6 ml of blood was drawn from the recurrent tarsal vein of the dog aseptically by using an 18-gauge needle. The blood was then transferred into two different tubes: EDTA - for preserving whole blood, and falcon tube -for serum isolation. Next, Giemsa staining was performed as outlined in the reference laboratory for animal trypanosomes diagnostic protocols of OIE (Desquesnes *et al*., 2013; Njiru *et al*., 2006). In short, a drop of whole blood was placed on a slide and spread using another slide to make a thick smear of an area approximately 2 cm in diameter. The slide was air dried and dehaemoglobinised by immersing in distilled water for a few seconds. It was then stained with Giemsa stain (0.4% pure commercial solution diluted 1:20 in phosphate- buffered saline, pH 7.2); the slide was immersed for 30 min, rinsed once in deionized water, and then rinsed well in tap water.

Afterwards, microscopic exploration was performed using BestScope BS-2022 biological microscope (Bestscope International Limited, Beijing, China). During the examination, trypomastigotes form of Trypanosoma were observed around blood cells in thin smears at 10X and 40X objective lenses (Fig. 1), and the case was diagnosed as canine trypanosomiasis. The biochemical and haematological examination of blood was then assessed to confirm our diagnosis of canine trypanosomiasis.

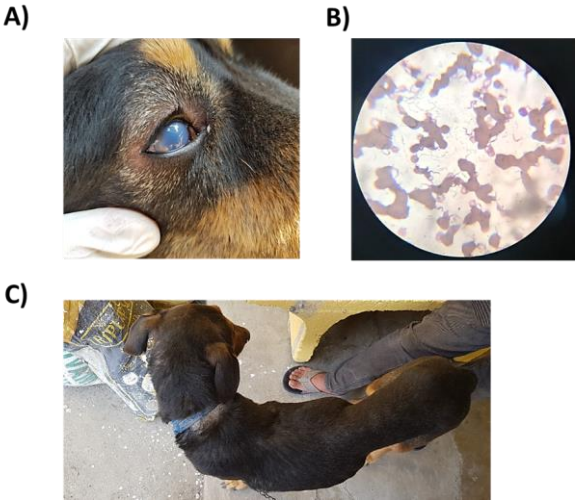

Fig 1. (A) Corneal Opacity observed in infected dog causing partial blindness of right eye, (B) Trypomastigotes forms of Trypanosoma in blood smear observed under the microscope (10X), and (C) Canine trypanosome infected mixed breed dog before treatment.

In laboratory examination, the CBC of the dog showed low Hb count and PCV indicating anaemia. Hb count was as low as 7 g/dL and PCV was also low at 21 % (Table 1). This explained the delayed capillary refill time and pale mucous membranes during physical examination (Claus, 2013). WBC count was high mainly because of elevated neutrophil count. Both BUN and Creatinine levels in

the blood were elevated and blood glucose level was decreased suggesting hypoglycaemia. The CBC and GHP were repeated 7 days after 1st treatment to track the progression of treatment. These indicators improved within the first week and were within the normal range after two weeks of treatment (Table 1).

Table 1. Blood report of the dog infected with Canine Trypanosoma, with the CBC and GHP parameters at day 0 and day 7 after treatment.

S. No	Parameter	Unit	Day 0	Day 7	Reference Value
1	Hemoglobin (Hb)	g/dL	7	9	11.9–18.9
2	Packed cell Volume (PCV)	%	21	27	35–57
3	White blood cell (WBC)	K/µL	23	15	5.0–14.1
4	Platelet Count	K/µL	50	170	211–621
	Differential Count				
6	Neutrophil	%	90	85	58–85
7	Lymphocyte	%	13	09	8–21
8	Eosinophil	%	01	01	0–9
9	Monocyte	%	00	00	2-10
10	Basophil	%	00	00	0-1
	Biochemical Tests				
11	Blood Urea Nitrogen (BUN)	mg/dl	63	20	7-30
12	Creatinine	mg/dl	1.9	1.1	0.5-1.8
13	Glucose	mg/dl	66	86	70-130

TREATMENTS AND DISCUSSION

After confirmatory diagnosis, supportive treatment was provided for convulsion, dehydration and fever. The fever subsided by the next day. Fluid therapy with Ringer's lactate solution (RL) was shifted to normal saline. The case was successfully treated by two doses of diminazene aceturate 7 days apart at the dose rate of 5 mg/kg body weight deep IM. The treatment showed good clinical improvement. A blood smear examination after a week was negative for Trypanosoma. Blood parameters improved after a week.

As prophylactic vaccination against canine trypanosomes has not been successful due to antigenic variation of the parasite, chemotherapy after infection with potent antiprotozoal is recommended. Diminazene aceturate is a diverse antiprotozoal drug and the drug of choice in canines against diseases such as Babesiosis and Trypanosomiasis. Diminazene aceturate has been used successfully to treat canine trypanosomiasis in German Shepherds (Panigrahi *et al*., 2015) and several other animal species (Ramesh et *al*., n.d.). The trypanocidal action of diminazene aceturate is by binding to kinetoplast DNA (kDNA) thereby inducing complete and irreversible loss of (kDNA) in certain strains of trypanosomes (Riou and Benard, 1980). Diminazene aceturate at 3.5 mg/kg body weight intramuscularly as single or multiple doses at a week apart is recommended as a therapeutic regimen by OIE. However, this approach was found to have a variable response (Hellebrekers and Slappendel, 1982). Suramin at 70 mg IV in 100 ml 0.9% NaCl TID every third day is also reported to successfully remove parasitaemia in canines (Defontis *et al*., 2012). Diminazene aceturate is mostly administered parenterally and predominantly bound to plasma with an absorption half-life in dogs 0.18 hours and a terminal half-life of 27.5 hours (Miller *et al*., 2005). The prognosis after early treatment by either of these drugs is favourable. Supportive therapy including intravenous fluids, and antipyretics are advised for canine trypanosomiasis (Behera *et al*., 2017). However, prolonged administration of this drug is associated with toxic effects on the nervous system and death in some cases (Abaru *et al*., 1984).

The dog had elevated WBC (23 K/µL) and reduced platelet count (50 K/µL). The change in WBC is mainly due to neutrophilia and lymphocytosis. Host cell apoptosis may also contribute to the increased production of WBC. Both neutrophil count and lymphocyte count showed lowered values after treatment. Lymphocyte count plays an active role in the immunopathogenesis of Trypanosomes. Additionally, in our study, we also found a lower level of platelets in Trypanosoma infected dog. This finding was aligned with previous studies which observed the low platelet count in trypanosomiasis (Marcondes *et al*., 2000; Mabbott and Sternberg, 1995). The lower platelet count is due to the pooling of blood in the spleen, removal of platelets by mononuclear phagocytic system and increased 'consumption' of platelets by disseminated intravascular coagulation reaction (Aster, 1966). The low platelet count and increased platelet volume indicated increased destruction of platelets by toxic products emanating from the trypanosomes. The dog

showed increased BUN and creatinine levels. This may be due to a condition that causes a decrease in the flow of blood to the kidneys such as dehydration (Blood Urea Nitrogen (BUN) - Understand the Test, n.d.). The value indicated towards the impaired function of the kidney in this case of Trypanosomiasis could be attributed to glomerulonephritis, excessive protein catabolism and febrile conditions which are common manifestations of this condition, presumably acting together to elevate BUN (Littman, 2011).

CONCLUSIONS

Canine trypanosomiasis is caused by a common pathogenic protozoan in Kathmandu, Nepal. Gradual loss of condition, renal impairment and ocular involvement seem to be consistent signs of chronic canine trypanosomiasis. Intermediate fly hosts must be controlled, and veterinarians should consider this disease in differential diagnoses in the study area. Clinical signs with blood profile reports and a wet blood smear can be used to diagnose the disease and diminazene aceturate can be successfully used to treat the disease.

REFERENCE

Abaru, D. E., Liwo, D. A., Isakina, D., and Okori, E. E. (1984). Retrospective long-term study of effects of Berenil by follow-up of patients treated since 1965. *Tropenmedizin Und Parasitologie*, **35**(3), 148–150.

Aster, R. H. (1966). Pooling of platelets in the spleen: Role in the pathogenesis of hypersplenic" thrombocytopenia. *Journal of Clinical Investigation*, **45**(5), 645–657. https://doi.org/10.1172/JCI105380.

Behera, S. K., Sarma, K., Behera, P., and Ayub Ali, M. (2017). Therapeutic management of trypanosomosis with ophthalmic involvement in a dog. *Journal of Parasitic Diseases: Official Organ of the Indian Society for Parasitology*, **41**(4), 1162–1165. https://doi.org/10.1007/s12639-017-0953-z.

Bhatia, B. B., Pathak, K. M. L., Juyal, P. D., and Kalyani Publishers. (2018). *Textbook of veterinary parasitology: (Based on the outline of Courses Approved by the Veterinary Council of India)*. Kalyani Publishers.

Blood Urea Nitrogen (BUN)—Understand the Test. (n.d.). Retrieved September 30, 2020, from https://labtestsonline.org/tests/blood-urea-nitrogen-bun

Claus, M. A. (2013). Veterinary Emergency and Critical Care Procedures, 2nd Edition—Edited by Timothy B. Hackett, Elisa M. Mazzaferro. *Journal of Small Animal Practice*, **54**(12), 676–676. https://doi.org/10.1111/jsap.12073

da Costa, A. P., Costa, F. B., Soares, H. S., Ramirez, D. G., de Carvalho Mesquita, E. T. K., Gennari, S. M., and Marcili, A. (2015). *Trypanosoma cruzi* and *Leishmania infantum chagasi* Infection in Wild Mammals from Maranhão State, Brazil. *Vector-Borne and Zoonotic Diseases*, **15**(11), 656–666. https://doi.org/10.1089/vbz.2015.1771

Defontis, M., Richartz, J., Engelmann, N., Bauer, C., Schwierk, V. M., Büscher, P., and Moritz, A. (2012). Canine Trypanosoma evansi infection introduced into Germany. *Veterinary Clinical Pathology*, **41**(3), 369–374. https://doi.org/10.1111/j.1939-165X.2012.00454.x

Desquesnes, M., Dargantes, A., Lai, D.-H., Lun, Z.-R., Holzmuller, P., and Jittapalapong, S. (2013). *Trypanosoma evansi* and Surra: A Review and Perspectives on Transmission, Epidemiology and Control, Impact, and Zoonotic Aspects. *BioMedical Research International*, *2013*, 1–20. https://doi.org/10.1155/2013/321237

Eloy, L., and Lucheis, S. (2009). Canine trypanosomiasis: Etiology of infection and implications for public health. *Journal of Venomous Animals and Toxins Including Tropical Diseases*, **15**(4), 589–611. https://doi.org/10.1590/S1678-91992009000400002

Hellebrekers, L. J., and Slappendel, R. J. (1982). Trypanosomiasis in a dog imported in the Netherlands. *Veterinary Quarterly*, **4**(4), 182–186. https://doi.org/10.1080/01652176.1982.9693860

Jaiswal, A. K., Sudan, V., . N., and Verma, A. K. (2015). Insight into Trypanosomiasis in Animals: Various Approaches for its Diagnosis, Treatment and Control: A Review. *Asian Journal of Animal Sciences*, **9**(5), 172–186. https://doi.org/10.3923/ajas.2015.172.186

Kjos, S. A., Snowden, K. F., Craig, T. M., Lewis, B., Ronald, N., and Olson, J. K. (2008). Distribution and characterization of canine Chagas disease in Texas. *Veterinary Parasitology*, **152**(3–4), 249–256. https://doi.org/10.1016/j.vetpar.2007.12.021

Kl, P., Pm, K., Vc, R., and Ch, S. (2013). Prevalence of canine trypanosomiasis in certain areas of Andhra Pradesh. *Journal of Parasitic Diseases : Official Organ of the Indian Society for Parasitology*, **39**(2), 238–240. https://doi.org/10.1007/s12639-013-0326-1

Littman, M. P. (2011). Protein-losing Nephropathy in Small Animals. *Veterinary Clinics of North America: Small Animal Practice*, **41**(1), 31–62. https://doi.org/10.1016/j.cvsm.2010.09.006

Mabbott, N., and Sternberg, J. (1995). Bone marrow nitric oxide production and development of anemia in Trypanosoma brucei-infected mice. *Infection and Immunity*, **63**(4), 1563–1566. https://doi.org/10.1128/IAI.63.4.1563-1566.1995

Marcondes, M. C. G., Borelli, P., Yoshida, N., and Russo, M. (2000). Acute Trypanosoma cruzi infection is associated with anemia, thrombocytopenia, leukopenia, and bone marrow hypoplasia: Reversal by nifurtimox treatment. *Microbes and Infection*, **2**(4), 347–352. https://doi.org/10.1016/S1286-4579(00)00333-6

Maxfield, L., and Bermudez, R. (2020). Trypanosomiasis (Trypansomiasis). In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK535413/

Miller, D. M., Swan, G. E., Lobetti, R. G., and Jacobson, L. S. (2005). The pharmacokinetics of diminazene aceturate after intramuscular administration in healthy dogs. *Journal of the South African Veterinary Association*, **76**(3), 146–150. https://doi.org/10.4102/jsava.v76i3.416

Njiru, Z. K., Constantine, C. C., Masiga, D. K., Reid, S. A., Thompson, R. C. A., and Gibson, W. C. (2006). Characterization of Trypanosoma evansi type B. *Infection, Genetics and Evolution*, **6**(4), 292–300. https://doi.org/10.1016/j.meegid.2005.08.002

Panigrahi, P. N., Mahendran, K., Jena, S. C., Behera, P., Mahajan, S., Arjun, K., and Dey, S. (2015). Trypanosoma evansi infection in a German shepherd dog—Apparent successful treatment using serial low dose of diminazene aceturate. *Veterinary Parasitology: Regional Studies and Reports*, **1–2**, 70–74. https://doi.org/10.1016/j.vprsr.2016.03.003

Ramesh, P., Chowdary, C. S. R., and Chaitanya, Y. (n.d.). *Diagnosis and Treatment of Canine Trypanosomiasis – A Case Study*. 7.

Riou, G., and Benard, J. (1980). Berenil induces the complete loss of kinetoplast DNA sequences in Trypanosoma equiperdum. *Biochemical and Biophysical Research Communications*, **96**(1), 350–354. https://doi.org/10.1016/0006-291X(80)91221-8

Rjeibi, M. R., Ben Hamida, T., Dalgatova, Z., Mahjoub, T., Rejeb, A., Dridi, W., and Gharbi, M. (2015). First report of surra (Trypanosoma evansi infection) in a Tunisian dog. *Parasite (Paris, France)*, **22**, 3. https://doi.org/10.1051/parasite/2015004

Rosypal, A. C., Cortés-Vecino, J. A., Gennari, S. M., Dubey, J. P., Tidwell, R. R., and Lindsay, D. S. (2007). Serological survey of Leishmania infantum and Trypanosoma cruzi in dogs from urban areas of Brazil and Colombia. *Veterinary Parasitology*, **149**(3–4), 172–177. https://doi.org/10.1016/j.vetpar.2007.08.004

Soulsby, E. J. L. (1982). *Helminths, Arthropods and Piotozoa of Domesticated Animals*. Bailliere Tindall.