

CASE REPORT:

A CLINICAL NOTE ON CANINE TRYPANOSOMIASIS

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ABSTRACT: Trypanosomes are widely distributed extra erythrocytic protozoan of animals. We reported a *Trypanosomes* infection from the blood of a bulldog. An 18 months old male was presented in the Pet Hospital of RCVetS, Lahore, Pakistan with a history of anorexia, emaciation, and pyrexia. Clinical examination of the dog indicated pale mucosa due and increase capillary refill time. Microscopic examination of blood films confirmed the *Trypanosomes* infection. Hematological analysis of the blood of the dog indicated a decrease in Total erythrocyte count, hemoglobin concentration, and Pack cell volume except for erythrocyte sedimentation rate. Differential leukocyte count revealed all parameters were unremarkable except Neutropenia, Eosinophilia, Lymphocytosis, and Thrombocytopenia. Serum analysis showed hyperproteinemia, elevated bilirubin, Blood urea nitrogen (BUN), Hypoglycaemia, and a decreased level of Gamma-glutamyl transferase (GGT). This directed us to work on heamo parasites in companion animals.

Keywords: Blood Parasitism, Dogs, Companion animals, *Trypanosomes*, Pet diseases.

Statement of novelty: This report highlights a very rare condition of *Trypanosoma* in pet dogs. The treatment and diagnosis of this haemo-parasite is not common practice. This condition is not reported in the past.

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INTRODUCTION

Trypanosomiasis is a globally prevalent vector-borne haemoprotozoal infection of all domesticated, companion, and wild animals. This infection is transferred mechanically by hematophagous flies of the genus *Stomoxys*, *Tabanus*, and *Glossina* sp. (Desquesnes et al., 2013). The vector flies only needs to transfer blood containing the infectious *Trypanosomes* sp. from one animal to another through the bite. In canines trypanosomiasis, the characteristic finding includes depression, emaciation, anorexia, diarrhea, anemia, conjunctivitis, intermittent fever, swelling of limbs, neurological symptoms, enlargement of lymph nodes, and occasionally corneal opacity (Ramesh, Chowdary, & Chaitanya, 2016). *Trypanosoma* produces a hyperacute and acute infection, If untreated, animals may lead to death (Asif et al., 2020; Rashid, Rasheed, & Hussain, 2008). During the infectious life cycle, the Trypomastigote form of parasite immediately enters the host cells, sub-clinically proliferates and escapes the immune system (Ahmed, 2018), and spreads through the host body mainly inside macrophages. Symptomatic parasitemia develops within 3-5 days post-infection (Rashid et al., 2008).

In Pakistan, this type of parasitemia is not frequently reported in canines except few reports (Asif et al., 2020; Rashid et al., 2008). Lahore is the provincial capital of Punjab, Pakistan, which exerts a strong cultural impact at the national level, a large number of its tenants keep dogs as game animals, pets, and guard animals. Taking into consideration the growing trend of dog keeping, this study aims to report canine trypanosomiasis to update the existing knowledge of canine trypanosomiasis in Pakistan in general and specifically in Lahore.

MATERIALS AND METHODS

Trypanosomiasis was diagnosed in a bulldog (18 months old) that was presented to the in the Pets Hospital, Riphah College of Veterinary Sciences, Lahore, on 26, October 2021. The owner narrates the history that the dog being kept with other domestic animals in a rural locality near a water pond in Lahore, Pakistan. Animal revealed non-specific clinical signs of 7% dehydration, high emaciation, pale buccal mucosa, and conjunctiva. The rectal temperature was 103.5 °F. On auscultation, heartbeat was 128 beats per minute (bpm) and respiration

rate was 12 breaths per minute. Live body weight of the dog was 16 Kilograms. Lymph nodes of the animal were not enlarged, however, a blood smear examination was performed (because of high incidence of canine babesiosis in Pakistan). Test results were found to be negative for *Babesia* spp. but there were high population of *Trypanosoma* spp. (Fig. 1&2). A 3 ml blood sample was drawn aseptically through cephalic vein in a sterile syringe and transferred to a test tube containing EDTA (Ethylenediaminetetraacetic acid) as an anticoagulant (Asif et al., 2020) and then used to make smear which were fixed on glass slide, stained by field staining and Giemsa staining respectively and examined under optical microscope at 100x oil immersion as per standard procedure of the Department of Pathology, RCVetS, Lahore, Pakistan. The blood film contained flagellate protozoans. The morphology of protozoa included undulating membrane, kinetoplast, central nucleus, and corkscrew shape Trypomastigotes resembling *Trypanosoma*. *Trypanosoma* was detected in blood smear can be seen in (Figure.1 & Fig.2). Haematological analysis including complete blood count (Battistoni et al., 2016), differential leukocyte count, and Serum Chemistry analysis. Hematological analysis of the sample performed by using URIT-2900 PLUS automatic blood cell analyzer/hematology analyzer revealed hypochromic anemia with decreased Erythrocyte count (3.01), Haematocrit value (23.8%) and Haemoglobin (7.9) shown in Table 1 (Sahar, Akhtar, Bilal, & Rana, 2012). Upon Differential leukocyte count, all parameters were unremarkable except Neutropenia (54.5), Eosinophilia (12%), Lymphocytosis (23.5%) and Thrombocytopenia ($169 \times 10^3/\mu\text{L}$) shown in table 2 (Rjeibi et al., 2015; Battistoni et al., 2016). MICROSYSTEM 300 Semi-Automatic Chemistry Analyser was used for serum analysis showed hyperproteinemia, elevated bilirubin, BUN, Hypoglycemia and decrease level of GGT shown in (Table 3)

This case was investigated through a detailed clinical investigation, microscopic examination/analysis of wet blood films and hematology profile. As a therapeutic regimen, a single dose of Diaminazine aceturate (3 mg/kg body weight) was administered intramuscularly along with the supportive fluid remedy.

RESULTS AND DISCUSSION

Trypanosomiasis is a widely distributed haemoparasite infection of animals. (Herrera et al., 2005), trypanosomiasis is considered a multi-species animal disease that can transmit through the biting of vectors flies (Luckins & Dwinger, 2004). In Pakistan, few

reports are available on this type of parasitemia from dogs (Asif et al., 2020; Rashid et al., 2008).

In the recent study, the prevalence of trypanosomiasis was detected in a male bulldog, kept with other domestic animals near a water pond for guarding purpose. This articulation is upheld by the findings of (Greene, 2006) who this stated that spread of this infection take place fundamentally near to water bodies by hosts and vector flies. Moreover, our findings were also parallel to the report of (Singh, Kalra, Gupta, & Nauriyal, 1993) who recorded sub-clinical trypanosomiasis in dogs kept near dairy animals for the duration of the rainy season. In the present study, anorexia, in-appetence, gradual weight loss, depression, pyrexia, swollen lymph nodes, corneal opacity, epistaxis, pale mucous membranes, and upon physical examination high capillary refill time were observed. Similar findings were recorded by Ramesh et al. (2016) and Rashid et al. (2008).

In this case, microscopic examination of stained blood smears showed of trypomastigotes stage (Fig. 1 & 2). (Asif et al., 2020; Irwin & Jefferies, 2004; Ramesh et al., 2016) also used blood smears as a diagnostic tool to confirm the presence of infection in dogs. Hematological analysis showed anemia, thrombocytopenia were recorded which are in accordance with reports of (Rjeibi et al., 2015). Hyperproteinemia, elevated bilirubin, BUN, and decrease level of GGT and Hypoglycemia were also observed in this study (Ramesh et al., 2016). In our observation anemia was a constant sign as stated earlier in various hosts infected with *Trypanosoma evansi* due to hemolysis, erythrophagocytosis, haemo-dilution, and a lower concentration of erythropoiesis as also observed by Jaktar and Purohit, (1971). In this study, neutropenia and relative lymphocytosis were noticed which are in line with results of the research of Aquino et al. (2002) and Ramesh et al. (2016).

Infested animals were treated with diminazine aceturate at a dose of 3.5 mg/kg of body weight deep Intramuscular, Meloxicam @ 0.5 mg/kg through sub/cut, dextrose 20% @ 5 ml/kg Intravenous followed by Dextrose normal saline until dehydration was corrected and oral hematinic supplementation (Fe-folate), (Wheeler) was given for two weeks to counter anemia. Clinical recovery was noted during the second week of therapy (recovery from pale mucosa, vanishing of corneal opacity). A single dose of diminazene aceturate is suitable for thorough suppression of parasitemia on the second day of therapy. Rashid et al. (2008) also reported that diminazene diacetate administered intramuscular once @ 3.5 mg/kg was effective in trypanosome infections in dogs as treated dogs showed clinical improvement after and disappearance of clinical signs after the treatment.

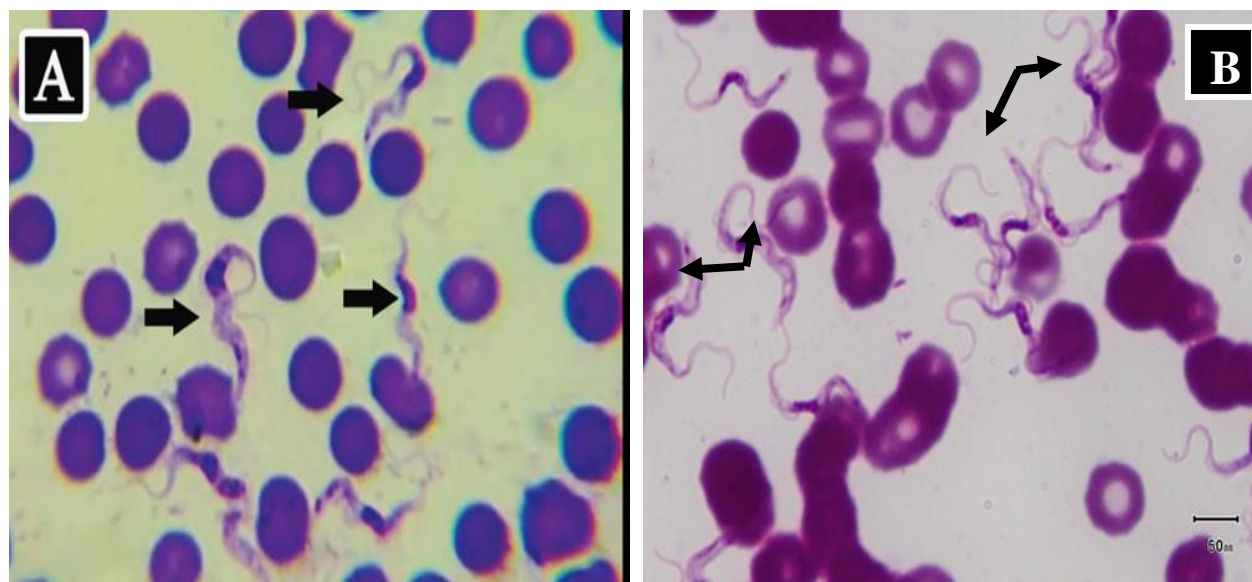


Fig 1. Blood film showing numerous Trypanosoma (A&B Arrows, field stain) (100x oil immersion)

Table 1: Erythrocyte Count and Indices.

Parameters (SI unit)	Results	Reference Range*
RBC ($\times 10^6/\mu\text{L}$)	3.01	4.95-7.87
PCV (%)	23.8	35-57
Haemoglobin (g/dL)	7.9	11.9-18.9
ESR (mm/h)	16	0-10
MCV (fL)	79.07	66-77
MCH (pg)	26.25	21-26.2
MCHC (g/dL)	33.19	32-36.3

*All reference values are taken from Susan E. Feilder, 2015.

Table 2: DLC Values

Parameters (SI unit)	Results	Reference Range*
TLC ($\times 10^3/\mu\text{L}$)	5.2	5-14.1
Neutrophils (%)	54.5	58-85
Eosinophil's (%)	12	0-9
Basophils (%)	1	0-1
Monocytes (%)	9	2-10
Lymphocytes (%)	23.5	8-21
Neutrophils ($\times 10^3/\mu\text{L}$)	2.83	2.9-12.0
Eosinophil's ($\times 10^3/\mu\text{L}$)	0.62	0-1.3
Basophils ($\times 10^3/\mu\text{L}$)	0.05	0-0.14
Monocytes ($\times 10^3/\mu\text{L}$)	0.68	0.1-1.4
Lymphocytes ($\times 10^3/\mu\text{L}$)	1.01	0.4-2.9
Platelet count ($\times 10^3/\mu\text{L}$)	169	211-621

*All reference range is taken from Susan E. Feilder, 2015.

Table 3: Result of Serum Chemistry Analysis.

Parameters	Results	Reference Range
Urea (g/L)	0.8	0.2-0.5
Creatinine (mg/L)	8.6	10-20
ALT (μL)	73.1	21-102
AST (μL)	63.5	23-66
GGT (μL)	<5	<10
Total Protein (g/L)	79.3	54-71
Bilirubin	0.49	0.1-0.3
Glucose	59.3	70-138

Conclusions: Dogs kept as pets especially near water ponds/sources may have a higher risks of *Trypanosoma* infestation due to vectors (fly) activity, especially during the rainy season. Enlargement of lymph nodes, pyrexia, and anemia are consistent findings, while corneal opacities and neurological symptoms may occur in the chronic form of the disease. A single dose of diaminisin aceturate can help in eliminating infective forms of trypanosomal organisms on the second day of therapy. Keeping view of present investigation heamoparasites are also prevalent in companion animals. Hence future work is needed on blood parasitism in canines

Ethical statement: Blood samples were collected according to the ethical standards of the Research Council of the University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan. Verbal consent was obtained from the owners before involvement in the study. Competing interests

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