



Peripheral Blood Leucocyte Apoptosis in Two Dogs Infected with *Trypanosoma congolense*

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INTRODUCTION

Blood leucocyte apoptosis in the trypanosome-infected natural hosts is yet to be documented and recognized as a feature of trypanosomiasis. We provide evidence of marked peripheral blood leucocyte apoptosis in two cases of dogs severely infected with *Trypanosoma congolense*. It is expected that this case report will prompt more investigations into trypanosome-induced leucocyte apoptosis, especially the identification of trypanosome apoptotic factors and their role in the pathogenesis of the disease. In addition, these case reports underline the importance of a thorough differential diagnosis in cases of suspected poisoning in exotic breeds of dog.

CASE REPORTS

A 6.5 year-old female Alsatian dog was brought to the Veterinary Teaching Hospital (VTH) Clinic, University of Ibadan, Nigeria in a comatose state and with complaint of sudden loss of activity. On physical examination, the mucous membranes were of normal colour. The rectal temperature was 39.2° C and the pulse 108b/min (within reference value). A tentative diagnosis of poisoning was made. Blood sample was collected for haematology and the dog placed on oxygen. The animal died the following day before any specific treatment could be initiated.

The haemogram showed mild hyperproteinaemia, borderline normocytic normochromic anaemia, moderate leucocytosis with a moderate left shift neutrophil and mild lymphocytosis (Table 1). These suggest an inflammatory response with immune stimulation. There was also a mild thrombocytopenia confirmed on blood smear examination.

Light microscopy examination of blood smear revealed numerous *T. congolense* parasites (figure 1a.); an average of 2/100x oil immersion field. In addition, morphological changes characteristic of apoptosis were noted in the leucocytes. The leucocyte apoptotic changes were

cytoplasmic blebbing, shrunken cells with deeply basophilic cytoplasm, condensed (figure 1b.) and sometimes fragmented nuclear chromatin (figure 1c, d & e.). Some chromatins were condensed and perinuclearly located (figure 1c & e.). A few apoptotic bodies (figure 1f.) were also found. Most of the apoptotic cells were lymphocytes (13/100 WBC) and a few

neutrophils (2/100 WBC) although a few cells with well-advanced apoptotic changes were not readily identified with a particular cell line. A few binucleated lymphocytes were also noticed. A diagnosis of severe acute trypanosomiasis was made.

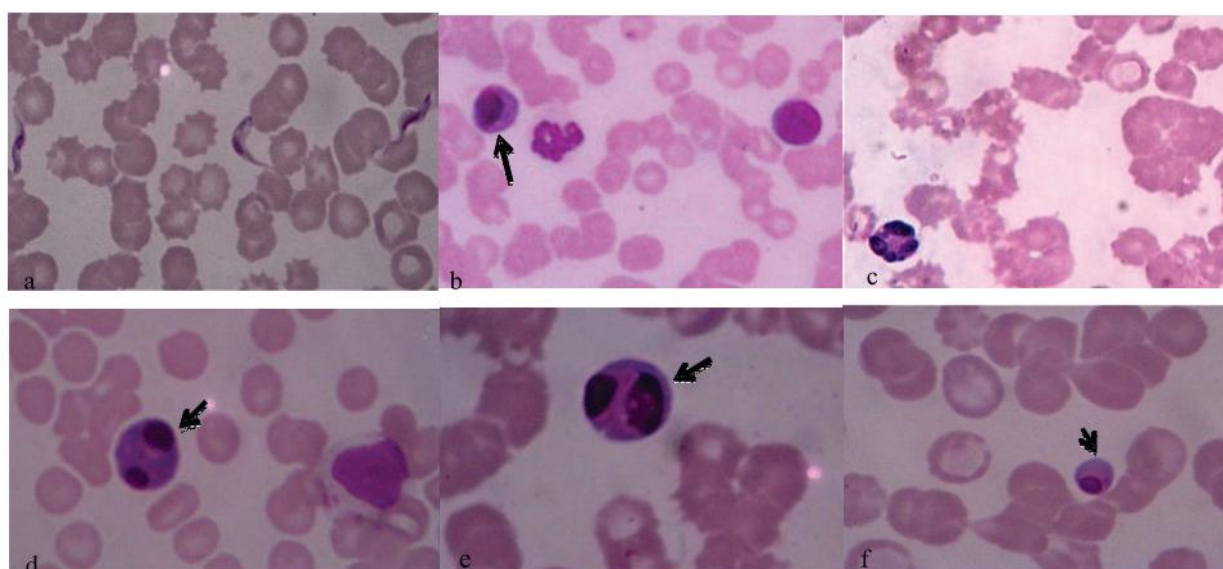


Figure 1. Blood smear from *T. congolense* infected dogs showing: a. Three *T. congolense* parasites, b. shrunken lymphocyte with condensed nuclear chromatin, c. a shrunken leucocyte with fragmented nuclear chromatin; d. shrunken lymphocyte with more basophilic cytoplasm and a condensed and fragmented nuclear chromatin; e. apoptotic lymphocyte showing condensed and fragmented nuclear chromatin pushed to the nuclear wall; f. apoptotic body. (Giemsa stain; x 1000 original magnification)

Another female Alsatian dog, 1.5 year-old was brought to the same clinic very weak and recumbent. The owner complained that the bitch had not been active, slightly weak and off feed for a day. He owned three (3) dogs but one died of trypanosomiasis two weeks earlier. On clinical examination, the rectal temperature was 39.7° C (slightly pyrexia) and the heart rate 120 beats/min (within reference range). The clinician suspected it was also trypanosomiasis and blood was collected for haematology. The haemogram showed severe anaemia, and thrombocytopenia (Table 1), while the leucocytic values were within reference range. Blood smear examination revealed abundant *T.*

congolense parasites, at an average of 2/100x oil immersion field. In addition, a few apoptotic lymphocytes (4/100 WBC) and neutrophils (2/100 WBC) were recorded. These apoptotic cells had similar morphological characteristics described in the previous dog above. The case was confirmed to be severe trypanosomiasis. Subsequently, the dog was treated using 3.5mg/kg of Berenil (diaminazine aceturate) and multivitamins injections. The blood parasites were cleared, the animal recovered and the haemogram 10 days post-treatment showed remarkable improvement (Table 1) with no apoptotic cells.

DISCUSSION

This report documents the first case of peripheral blood leucocyte apoptosis in canine *T. congolense* infections.

There have been renewed interests in understanding the relevance of blood cell apoptosis in protozoan infections such as with *P. falciparum* (Toure'-Balde' et al., 1996) and *T. cruzi* (Silva et al., 2007). Although, Happi et al. (2012) recently reported *T. brucei*-induced leucocyte apoptosis in experimentally infected rats and suggested that leucocyte apoptosis may contribute to the severity of leucopaenia and the disease, there is currently no documentation of an association between *T. congolense* infection and host cell apoptosis.

The trypanosome species observed in these cases are consistent with the report that dogs are mostly susceptible to *T. congolense* (Greene, 2006). Both cases also showed severe parasitaemia (an average of 2/100x oil immersion field). However, the first animal succumbed rapidly while the second one responded to treatment and survived. In addition, apoptosis of peripheral blood leucocytes is not easily observed in circulation as they are rapidly engulfed by adjacent monocytes (Savill and Fadok, 2000). Apoptotic leucocytes were readily observed in peripheral blood of the two animals. However, they were much higher in the first case (comatose dog) with a lymphocyte apoptotic index of 13% and a fatal outcome. This observation suggests that massive peripheral leucocyte apoptotic induction with trypanosoma infection

TABLE 1. Results of the haematology in two dogs with *T. congolense* infection

Parameters	Case1	Case 2		Reference*
		On presentation	Day10 post treatment	
PCV (%)	34	18	38	35-57
RBC (x10 ⁶ /µl)	5.19	2.24	6.52	4.95-7.87
MCV (fl)	66	80	66	66-77
MCHC (g/dl)	32	31	31	32-36
Platelet (x10 ³ /µl)	200	96	198	211-621
TWBC (x10 ³ /µl)	18.3	10	8.3	5.9-14.1
Seg. Neutrophils (x10 ³ /µl)	10.6	5.7	5.3	2.9-12.0
Band Neutr. (x10 ³ /µl)	0.9	0.5	0	0-0.45
Lymphocytes (x10 ³ /µl)	5.5	2.9	2.8	0.4-2.9
Monocytes (x10 ³ /µl)	0.9	0.7	0.1	0.1-1.4
Eosinophils (x10 ³ /µl)	0.4	0.2	0.1	0.0-1.3

*Haematology reference values from the 3rd Edition Veterinary Laboratory Medicine, Clinical Pathology, Duncan, Prasse and Mahaffey (1994).

indicate a poor prognosis. Nonetheless, further studies of more cases may provide factual evidence to this suspicion.

Contrary to what is expected, the high leucocyte apoptotic index did not appear to influence the total leucocyte counts. This is possibly because the haemopoietic/lymphopoietic organs were still able to respond adequately during the acute stage of the infection and or the immune stimulation induced by the presence of the pathogen that is manifested by lymphocytosis. In addition, the apoptotic cells might have been counted as normal nucleated cells in which case the total leucocyte count might remain unchanged until such apoptotic cells are removed from the circulation. In a sub-acute or chronic trypanosomal infection, pancytopenia is the usual hematological finding (Naessens, 2006). This might be due to exhaustion of the haemopoietic organs to replace cell loss as the decline in the total white blood cell (WBC) count during trypanosomiasis is due primarily to a significant reduction in the lymphocyte counts (Ezeokonkwo et al., 2010).

Furthermore, pancytopenia in trypanosomiasis has been reported to be closely associated with peak parasitaemia (Maxie et al., 1979). However, the mechanisms involved in the pancytopenia remain unclear. It will be necessary therefore to investigate the relationship between leucocyte apoptosis and leucopaenia given that peripheral blood leucocyte apoptosis is seldomly associated with African Animal Trypanosomiasis.

A mild thrombocytopenia, mild anemia and an inflammatory leucogram were observed in the first case, while the second case showed a severe regenerative anaemia and thrombocytopenia. The latter is consistent with the haematological findings of regenerative anaemia and thrombocytopenia of a German wire-haired pointer infected with *T. congolense* (Museux et al., 2011). It also emphasizes the fact that haemogram in trypanosomal infections is very variable, it may show macrocytic or normocytic anaemia, associated with neutropaenia and lymphopaenia or leucocytosis and thrombocytopenia (Greene, 2006) or anemia, thrombocytopenia, and leucopenia, with subsequent induction of immunosuppression (Taylor and Authie, 2004).

CONCLUSION

In conclusion, the cases presented in this report provide the first evidence of blood leucocyte apoptosis in natural host infected with *T. congolense* and point to the necessity to investigate the relationship between leucocyte apoptosis and leucopaenia in African Animal Trypanosomiasis. In addition, identification of leucocyte apoptotic factors in trypanosomiasis may provide important targets for the control of the disease. These cases also highlight the need to have a list of differential diagnoses for clinical signs of acute poisoning which includes severe canine trypanosomiasis in dogs, especially in endemic regions. They also stress the importance of blood smear

examination as it may facilitate the diagnosis of severe acute cases and allow clinicians to initiate prompt and appropriate treatment.

REFERENCES

- DUNCAN, J.R., PRASSE, K.W. and MAHAFFEY, E.A. (1994): *Veterinary Laboratory Medicine, Clinical Pathology* 3rd Edition. Iowa State University Press, Ames, Iowa: 235.
- EZEOKONKWO, R.C., EZEH, I.O., ONUNKWO, J.I., OBI, P.O., ONYENWE, I.W. and AGU, W.E. (2010): Comparative haematological study of single and mixed infections of mongrel dogs with *Trypanosoma congolense* and *Trypanosoma brucei brucei*. *Veterinary parasitology*. 173 (1-2): 48-54.
- HAPPI, N.A., MILNER JR., A.D. and ANTIA, E.R. (2012): Blood and tissue leucocyte apoptosis of in *T. brucei* infection in rats. *Journal of Neuroparasitology* Vol. 3, doi:10.4303/jnp/N12010, 10 pages.
- GREENE, C.E. (2006): *Trypanosomiasis. Infectious Diseases of the Dog and Cat*. 3rd edn. W.B. Saunders: 676-685.
- MAXIE, M.G., LOSOS, G.J. and TABEL, H. (1979): Experimental bovine trypanosomiasis (*Trypanosoma vivax* and *T. congolense*). I. Symptomatology and clinical pathology. *Tropenmedizin und Parasitologie*, 30 (3): 274-82.
- MUSEUX, K., BOULOUHA, L., MAJANI, S. and JOURNAUX, H. (2011): African *Trypanosoma* infection in a dog in France. *Veterinary Record*, 168: 590.
- NAESSENS, J. (2006): Bovine trypanotolerance: A natural ability to prevent severe anaemia and haemophagocytic syndrome? *International Journal for Parasitology*, 36: 521-528.
- SAVILL, J.S. and FADOK, V. (2000): Corpse clearance defines the meaning of cell death. *Nature*. 407: 787-788.
- SILVA, E.M., GUILLERMO, L.V., RIBEIRO-GOMES, F.L., DE MEIS, J., NUNES, M.P., SENRA, M.B., DOSREIS, G.A. and LOPES, M.F. (2007): Caspase inhibition reduces

lymphocyte apoptosis and improves host immune responses to *Trypanosoma cruzi* infection. *Eur. J Immunol.* 37(3): 738-746.

TAYLOR, K. and AUTHIE E.M-L. (2004): Pathogenesis of Animal Trypanosomiasis. The Trypanosomiasis. Eds. Mauldin I. Holmes P.H. and Miles M.A.: 331-354.

TOURE'-BALDE', A., SARTHOU, J.L., ARIBOT, G., MICHEL, P., TRAPE J.F., ROGIER, C. and ROUSSILHON, C. (1996): Plasmodium falciparum induces apoptosis in human mononuclear cells. *Infect. Immun.* 64:744-750.