Detection of human-infective trypanosomes in acutely-infected Jack Russel from Zambia's south Luangwa national park by loop-mediated isothermal amplification

B. Namangala[1], E. Oparaocha[2], B. Mubemba[1], C. Hankanga[3], L. Moonga[1], J. Ndebe[4], M. Lisulo[5], K. Kajino[6], K. Hayashida[6] and C. Sugimoto[6]

[1]Department of Paraclinical studies, School of Veterinary Medicine, University of Zambia, P.O. Box 32379, Lusaka, Zambia; [2]Show Grounds Veterinary Clinic, P.O. Box 30333 Lusaka, Zambia; [3]Department of Clinical Studies, School of Veterinary Medicine, University of Zambia, P.O. Box 32379, Lusaka, Zambia; [4]Department of Disease Control, School of Veterinary Medicine, University of Zambia, P.O. Box 32379, Lusaka, Zambia; [5]Department of Biomedical Sciences, School of Medicine, University of Zambia, P.O. Box 50110, Lusaka, Zambia; [6]Research Centre for Zoonosis Control, Hokkaido University, Sapproro, Japan.
E-mail: boniface_1020@yahoo.com; b.namangala@unza.zm

SUMMARY

A two-year old female Jack Russel dog, from Mfuwe, within Zambia's South Luangwa National Park (SLNP), was presented to a local Veterinary Clinic in Lusaka, exhibiting clinical signs of marked laboured breathing, lethargy and dullness. Further clinical examination revealed congested mucous membranes, rough hair coat, distended abdomen, enlarged lymph nodes and pyrexia of 41.5°C. A diagnosis of acute canine African trypanosomosis was made by microscopic examination of blood smear. Loop-mediated isothermal amplification (LAMP) analysis, using primers specifically targeting the human serum resistance-associated (SRA) gene, revealed a monolytic infection with *Trypanosoma brucei rhodesiense*. The dog died before treatment could be effected. Postmortem examination revealed profound hepatosplenomegally, marked congestion of kidneys, heart and lungs, ascites and hydrothorax. The potential public health implications of this infection are discussed.

Key words: Canine African trypanosomosis; Female Jack Russel dog; Sleeping sickness; South Luangwa National Park, SRA LAMP, *Trypanosoma brucei rhodesiense*.

INTRODUCTION

Dogs are affected by many arthropod-transmitted disease-causing organisms including *Babesia*, *Leishmania, Trypanosoma*, *Anaplasma, Ehrlichia, Dirofilaria* and *Dipylidium* spp that can also cause diseases in man (Dantas-Torres, 2008). As such, dogs have remained an important source of emerging and reemerging diseases in man throughout their long history of domestication (Dantas-Torres, 2008). African trypanosomes are mainly transmitted by tsetse flies (*Glossina* spp). Those that affect dogs include the African trypanosomes *Trypanosoma brucei* subspecies, *Trypanosoma congolense* and *Trypanosoma evansi* (Dantas-Torres, 2008; Matete, 2003; Gow *et al*., 2007; Eloy and Lucheis, 2009; Namangala *et al*., 2012). The disease caused by African trypanosomes in dogs may range from asymptomatic, chronic to acute fatal forms.

Although there are several reports of AAT in domesticated farm animals such as cattle and goats (Taylor, 1998; Konnai *et al*., 2008; Laohasinnarong *et al*., 2011), only few published reports of African trypanosome infections in dogs exist (Matete, 2003, Gow *et al*., 2007; Keck *et al.,* 2009; Museux *et al*., 2011). Interestingly, most of the CAT reports involve exotic breeds of dogs (Gow *et al*., 2007, Hooft, 2008; Museux *et al*., 2011; Namangala *et al*., 2012). Similarly, here we report a highly acute case of canine animal trypanosomosis (CAT) involving a female Jack Russell, an exotic breed of dog, which contracted the disease from the tsetse-infested South Luangwa National Park (SLNP). This report further highlights the acute nature of the disease caused by *T. b. rhodesiense* in exotic dog breed and the potential role dogs play as reservoirs of human-infective trypanosomes.

MATERIALS AND METHODS

Case history

In January 2012, the Show Grounds Veterinary Clinic in Lusaka recorded a case of CAT involving a two-year old female Jack Russel dog. The dog was from Mfuwe, SLNP, Mambwe district (Figure 1). This is a known tsetse-infested area within the Luangwa valley. The patient had a highly acute infection characterized by fulminate parasitaemia and nervous symptoms. Other clinical manifestations included anorexia, lethargy and dullness. Clinical examination further revealed enlarged lymph nodes, distended abdomen, pale mucous membranes, ocular mucopurrulent discharge and pyrexia of 41.5^{o}C, with laboured breathing.

Sample collection and microscopy

Blood was collected from the dog's cephalic vein into heparinised vacutainer tubes using a 5 ml syringe and microscopic examination of a wet

film carried out. Thin blood smears were prepared, stained with Giemsa as routine at 10% dilution for microscopic examination.

DNA extraction and Loop-mediated isothermal amplification (LAMP) reaction

Some of the blood collected was submitted to the University of Zambia, School of Veterinary Medicine, for identification of trypanosome species by LAMP. Briefly, DNA was eluted from the dried blood samples on FTA® Elute card by boiling three punched paper discs at 95°C for 30 minutes in an eppendorf tube containing 30 µl distilled water (Whatman FTA® Elute Cards, Whatman, UK). A LAMP reaction of 25 µl was performed using a Loopamp DNA Amplification Kit (Eiken Chemical, Tochigi, Japan) and the extracted DNA as template, as described by Thekisoe *et al.* (2007).

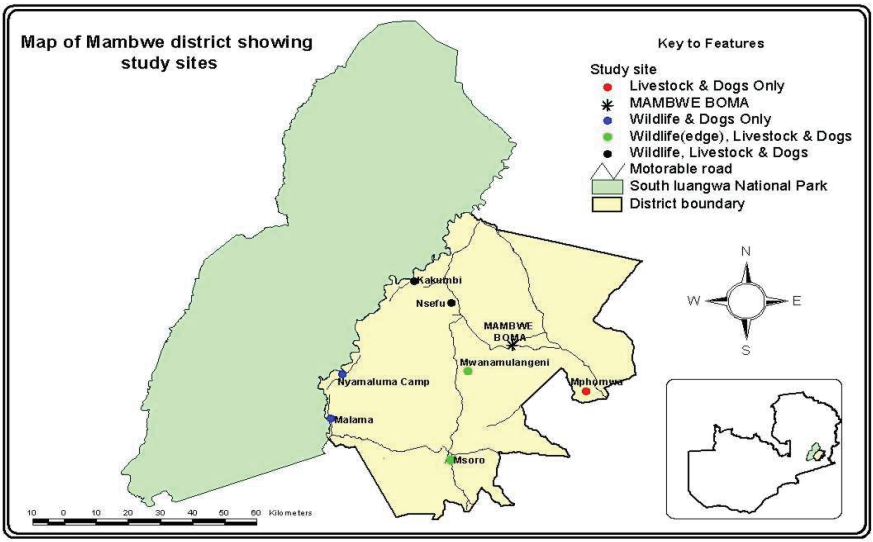

Figure 1. Map of South Luangwa National Park where the dog is reported to had contracted trypanosomosis.

Primers used were those recently described by Njiru *et al.* (2008) for the human serum resistance- associated (SRA) LAMP. The reaction mixture was incubated at 64°C for 30 minutes in a heat block (Dry Thermounit DTU 1B, TAIEC Co., Saitama, Japan) and then at 95°C for 2 minutes to terminate the reaction. The LAMP products were visualized using a transilluminator (WD, H19, Good design award Co., Japan).

RESULTS

Clinical examination of the dog revealed severe anaemia, as evidenced by the low packed cell volume (PCV) value of 12% found. Wet smear examination revealed the presence of several motile trypanosomes, which, together with the history, suggested that the dog was suffering from CAT. Microscopic examination of Giemsa-stained thin blood smear revealed characteristic features of bloodstream *Trypanozoon* trypomastigotes (Figure 2), suggesting that it belonged to *Trypanosoma brucei* complex. The patient died shortly after being brought to the clinic. Postmortem examination of the dead dog revealed marked enlargement of the liver and the spleen, severe congestion and oedema of the brain, kidneys and lungs, ascites and hydrothorax (Figure 3).

The resultant LAMP products are shown in Figure 4. The presence of *Trypanosoma brucei* subspp in dog blood, which was initially detected by microscopy, was confirmed by SRA LAMP, which further identified the subspecies of the trypanosomes as the human-infective *T. b. rhodesiense.*

DISCUSSION

In the present study, both microscopy and LAMP confirmed *T. brucei rhodesiense* as cause of a fatal infection in a Jack Russel, thus corroborating previous reports (Gow *et al.,* 2007; Hooft 2008; Museux *et al.,* 2011; Namangala *et al.,* 2012) that most cases of CAT are from exotic breeds. The infection was characterized by massive parasitosis that resulted in high fever, laboured breathing, nervous symptoms, anaemia, severe inflammatory and congestive reactions in visceral organs and the brain and death within three days after the initial clinical signs were observed. Indeed, members of the *T. brucei* complex are known to traverse vascular tissues, causing damage to various extravascular tissues (Horchner *et al*., 1985; Vershney *et al*., 1998), resulting in acute CAT (Matete, 2003), whereas infections caused by the strictly intravascular *T. congolense* are generally chronic in nature (Gow *et al*., 2007; Hooft, 2008). According to previous reports (Horchner *et al*., 1985; Abenga *et al*., 2005), indigenous dog breeds in tsetse-infested regions of sub-Saharan Africa seem to be trypanotolerant. CAT in such indigenous dogs is either subclinical or may be asymptomatic. Similarly, trypanotolerance has been documented in some indigenous West African cattle breeds such as the N'Dama (Taylor, 1998).

In the present report, CAT was initially detected by microscopy and later confirmed by SRA LAMP. The latter which is highly specific and sensitive, has the advantage over PCR of being simpler, rapid, cheaper and easier to perform as it only requires a heating device for incubation (Notomi *et al*.,

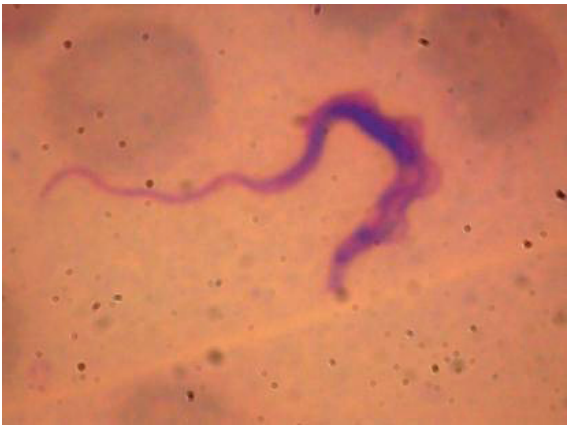

Figure 2. Giemsa-stained thin blood smear showing *Trypanosoma* brucei subspp (x 100) captured by a digital camera fitted onto the eyepiece.

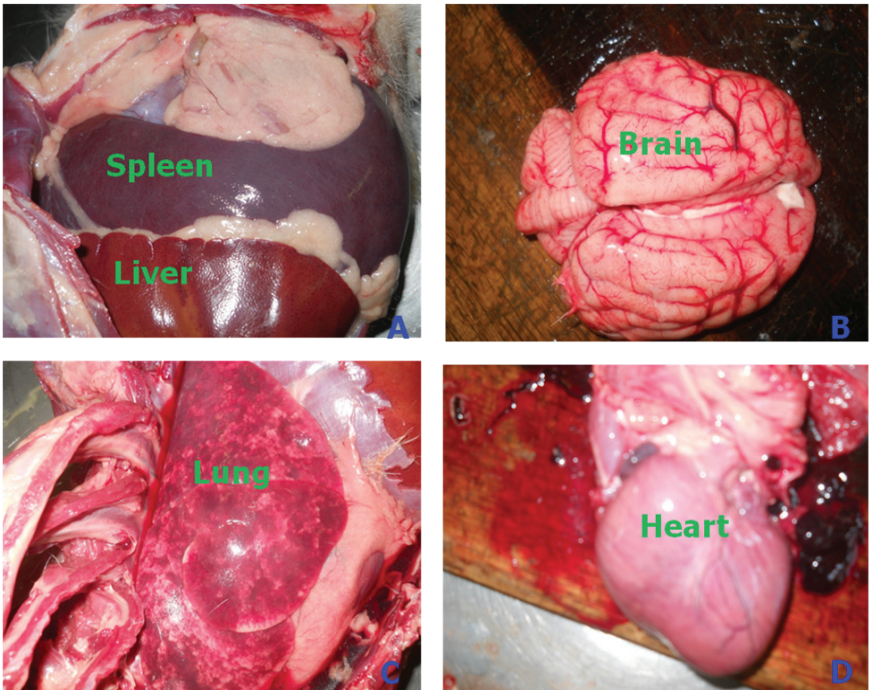

Figure 3. Postmortem findings of a dog (Jack Russel) that died from acute trypanosomosis showing hepatosplenomegally (A), inflamed and congested brain tissue (B), congested lungs (C) and rounded and congested heart (D).

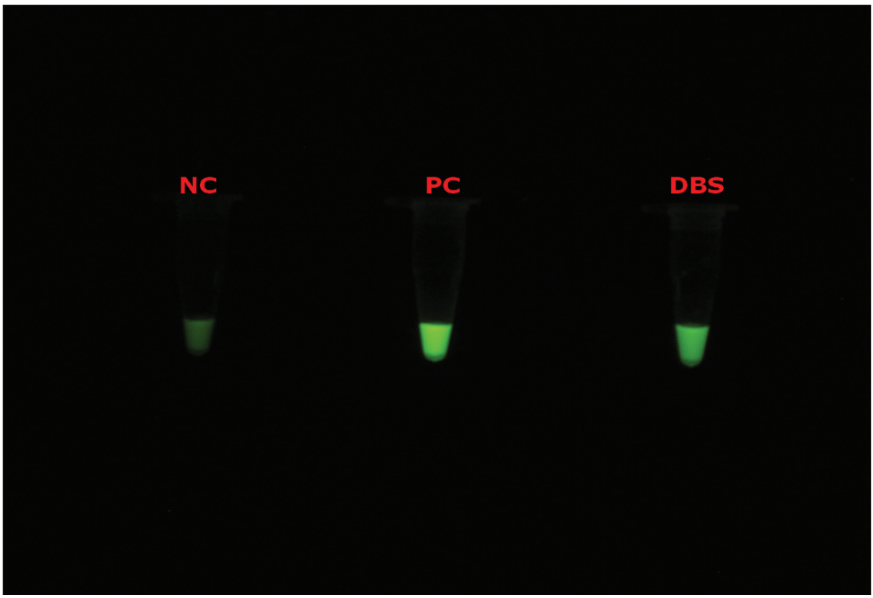

Figure 4. Visual appearance of results for human serum resistance-associated (SRA) LAMP for Trypanosoma brucei rhodesiense identification in dog (Jack Russel) blood sample. Loopamp Fluorescent detection reagent was added to the reaction mixture at the beginning of the assay. The reactions were incubated at 64oC for 30 minutes. In contrast to the light green background fluorescence in the negative samples, positive samples exhibit a bright fluorescent green colour when visualized under the transilluminator. NC: Negative control (distilled water); PC: Positive control (Trypanosoma brucei rhodesiense); DBS: dog blood sample.

2000) and may thus be more practical for resource-limited communities where Human African Trypanosomosis (HAT) is endemic. Furthermore, the large amount of DNA formed during a LAMP reaction allows visual detection of amplicons by naked eyes or through measurement of turbidity (Mori *et al*., 2001) or fluorescence (Poon *et al*., 2006).

Because the SRA gene defines *T. b. rhodesiense*, it provides unequivocal identification of that parasite (Xong *et al*., 1998, Gibson *et al*., 2002, Njiru *et al*., 2008). The finding of SRA gene in trypanosomes isolated from a dog in this report confirms the ability of dogs to act as a source of sleeping sickness (SS), as previously reported elsewhere (Matete, 2003; Njiru *et al*., 2008; Namangala *et al*., 2012). Although *T. b. rhodesiense* infection in dogs is acute, tsetse flies have a high chance of picking the disease during the blood meal because of the high

parasiteamia often associated with such infections. Of note, SRA-positive trypanosomes were also recently reported in an African buffalo in SLNP (Anderson *et al*., 2011). In aggregate, these observations are indicative of the risk of contracting SS by people living in or visiting SLNP. Indeed, at least 24 cases of SS were documented through passive surveillance at local clinics within Luangwa valleys between 2000 and 2007 (Mwanakasale and Songolo, 2011). Elsewhere, cattle (Welburn *et al*., 2001) and pigs (Njiru *et al.,* 2008) have also been reported to be reservoirs of the human-infective *T. b. rhodesiense*. Thus more detailed scientific investigations are needed to confirm the precise role dogs play in SS epidemiology in tsetse-infested regions such as Zambia.

ACKNOWLEDGEMENTS

Laboratory analysis was supported by the Center for Zoonosis Research, Hokkaido University, Faculty of Veterinary Medicine, Japan, through the fund under the project entitled "Surveillance studies of emerging and re-emerging zoonoses in Zambia".

REFERENCES

Abenga J, David K, Ezebuiro C, Lawani F. Observation on the tolerance of young dogs (puppies) to infection with *Trypanosoma congolense*. *Afric J Clin Exp Microb* 6: 28-32, 2005.

Anderson NE, Mubanga J, Fevre EM, Picozzi K, Eisler MC, Thomas R, Welburn SC. Characterization of the wildlife reservoir community for human and animal trypanosomiasis in the Luangwa Valley, Zambia. *PLoS Negl Trop Dis* 5: e1211. Doi.10.1371, 2011.

Dantas-Torres F. Canine vector-borne diseases in Brazil. *Parasit Vectors* 1: 25, 2008.

Eloy LJ, Lucheis SB. Canine trypanosomiasis: etiology of infection and implications for public health. *J Venom Anim Toxins incl Trop Dis* 15: 589-611, 2009.

Gibson W, Backhouse T, Griffiths A. The human serum resistance associated gene is ubiquitous and conserved in *T.b.rhodesiense* throughout East Africa. *Infect Genet Evol* 1: 207-214, 2002.

Gow AG, Simpson JW, Picozzi K. The first report of canine African trypanosomosis in the UK. *J Small Anim Pract* 48: 658-661, 2007.

Hooft EM. Canine African trypanosoma. *J Small Anim Pract* 49: 487, 2008.

Horchner F, Zillmann U, Metzner M, Schonefeld A, Hehlitz D. West African dogs as a model for research on trypanotolerance. *Trop Med Parasitol* 36: 257-258, 1985.

Keck N, Herder S, Kaba D, Solano P, Gomez J, Cuny G, Davoust B. Epidemiological study of canine trypanosomosis in an urban area of

Ivory Coast. *Parasite* 16: 305-308, 2009.

Konnai S, Mekata H, Odbileg R, et al. Detection of Trypanosoma brucei in field-captured tsetse flies and identification of host species fed on by the infected flies. *Vector Borne Zoonotic Dis* 8: 565-573, 2008.

Laohasinnarong D, Thekisoe OM, Malele I, et al. Prevalence of Trypanosoma sp. in cattle from Tanzania estimated by conventional PCR and loop-mediated isothermal amplification (LAMP). *Parasitol Res* 109: 1735-1739, 2011.

Matete GO. Occurrence, clinical manifestation and the epidemiological implications of naturally occurring canine trypanosomosis in western Kenya. *Onderstepoort J Vet Res* 70: 317-323, 2003.

Mori Y, Nagamine K, Tomita N, Notomi T. Detection of Loop-mediated isothermal amplification reaction by turbidity derived from magnesium pyrophosphate formation. *Biochem Biophys Res Commun* 289: 150-154, 2001.

Museux K, Boulouha L, Majani S, Journaux H. African Trypanosoma infection in a dog in France. Vet Rec 168: 590, 2011.

Mwanakasale V, Songolo P. Disappearance of human African trypanosomiasis transmission foci in Zambia in the absence of tsetse fly and trypanosomiasis control program over a period of forty years. *Trans Royal Soc Trop Med Hyg* 105: 167-172, 2011.

Namangala B, Oparaocha E, Inoue N, Mweene AS, Moonga L, Lisulo M and Sugimoto C. Detection of the human-infective Trypanosoma brucei rhodesiense in dog blood from Chiawa, Zambia. *UNZA-JST* 16: 9-14, 2012.

Njiru ZK, Mikosza AS, Armstrong T, Enyaru JC, Ndung'u JM, Thompson AR. Loop-mediated isothermal amplification (LAMP) method for rapid detection of Trypanosoma brucei rhodesiense. *PLoS Negl Trop Dis* 2: e147, 2008.

Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N, Hase T. Loop-mediated isothermal amplification of DNA. *Nucleic Acids Res* 28: E63, 2000.

Poon LL, Wong BW, Ma EH et al. Sensitive and inexpensive molecular test for Falciparum malaria: detecting Plasmodium falciparum DNA directly from heat-treated blood by Loop-mediated isothermal amplification. *Clin Chem* 52: 303-306, 2006.

Taylor KA. Immune response of cattle to African trypanosomes: protective or pathogenic? *Int J Parasitol* 28: 219-240, 1998.

Thekisoe OM, Kuboki N, Nambota A, et al. Species-specific loop-mediated isothermal amplification (LAMP) for diagnosis of trypanosomosis. *Acta Trop* 102: 182-189, 2007.

Vershney JP, Varshney VP, Dwivedi SK. Clinico-endochronological findings in clinical trypanosomiasis in dog. *J Vet Parasitol* 12: 143-144, 1998.

Welburn SC, Picozzi K, Fevre EM, Coleman PG, Odiit M, Carrington M, Maudlin I. Identification of the human-sensitive trypanosomes in animal reservoir of sleeping sickness in Uganda by means of serum-resistance-associated (SRA) gene. *Lancet* 358: 2017-2019, 2001.

Xong HV, Vanhamme L, Chamekh M, et al. A VSG expression site-associated gene confers resistance to human serum in Trypanosoma rhodesiense. *Cell* 95: 839-846,1998.