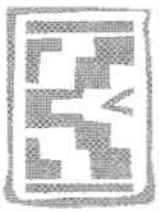

A comparative study on the clinical, parasitological and molecular diagnosis of bovine trypanosomosis in Uganda

J.W. MAGONA[1, 3], J.S.P. MAYENDE[3], W. OLAHO-MUKANI[3], P.G. COLEMAN[2], N.N. JONSSON[1], S.C. WELBURN[2], M.C. EISLER[1]

ABSTRACT

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The clinical, parasitological and molecular diagnosis of bovine trypanosomosis were compared using samples from 250 zebu cattle exposed to natural trypanosome challenge in Uganda. Clinical examination, molecular and parasitological diagnoses detected 184 (73.6%), 96 (38.4%) and 36 (14.4%) as diseased, respectively. The sensitivity and specificity of clinical examination were 87.5% and 35%, and 78% and 27% based on molecular and parasitological diagnoses, as gold standards, respectively. Of the 33, 3, 13 and 12 parasitological-positive cattle that had *Trypanosoma brucei, Trypanosoma congolense, Trypanosoma vivax* or mixed infections, 78%, 33%, 84% and 100% respectively manifested clinical signs. Of the 24, 89, 12, 3, 6 and 27 cattle detected by molecular diagnosis to have mixed infections, *T. brucei, T. vivax, T. congolense* forest-, Savannah- and Tsavo-type, 100%, 83%, 91%, 100%, 67% and 81% had clinical signs, respectively. In conclusion, treatment of cattle based on clinical examination may clear up to 87.5% or 78% of the cases that would be positive by either molecular or parasitological diagnosis, respectively. Under field conditions, in the absence of simple and portable diagnostic tools or access to laboratory facilities, veterinarians could rely on clinical diagnosis to screen and treat cases of bovine trypanosomosis presented by farmers before confirmatory diagnosis in diagnostic centres for few unclear cases is sought.

Keywords: Cattle, diagnosis, trypanosomosis

INTRODUCTION

The diagnosis of trypanosomosis relies on the detection of parasites using thin and thick blood smears, the haematocrit centrifuge technique (HCT) (Woo 1969) and phase contrast buffy-coat technique (BCT) (Murray, Murray & McIntyre 1977), and serology diagnosis using an antigen-detection ELISA (Nantulya & Lindqvist 1989) and antibody-detection ELISA (Luckin 1977; Hopkins, Chitambo, Machila, Luckins, Rae, Van den Bossche & Eisler 1998; Rebeski, Winger, Rogovic, Robinson, Crowther & Dwinger 1999). More recently polymerase chain reaction (PCR)-based techniques have been employed in detecting trypanosomes in bovine blood (Solano, Michel, Lefrancois, De la Rocque, Sidibe, Zoungrana & Cuisance 1999).

Direct parasitological techniques such as HCT, BCT, thick and thin blood films, have poor to fair

[1] University of Glasgow Veterinary School, Bearsden Road, Bearsden G61, 1QH, Glasgow, UK

[2] Centre for Tropical Veterinary Medicine, The University of Edinburgh, Easter Bush, Roslin, Midlothian, Scotland EH25 9RG, UK

[3] Livestock Health Research Institute, P.O. Box 96, Tororo, Uganda

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diagnostic sensitivity under field conditions. Unlike thick and thin blood films, recent findings revealed that BCT has a good diagnostic sensitivity (67–96 % for *Trypanosoma congolense* infection and 60–76 % for *Trypanosoma vivax* infection) under optimized conditions (Eisler, Lessard, Masake, Moloo & Peregrine 1998). Despite their low sensitivity, direct parasitological tests are suitable for field application and for diagnosis of individual cases. Serological tests for trypanosomosis may only be useful in providing information for targeting tsetse and trypanosomosis control (Hopkins *et al.* 1998) and for epidemiological studies. Unlike the antibody-detection ELISA, the antigen-detection ELISA has been reported to have a low specificity and sensitivity (Desquesness 1996; Eisler *et al.* 1998). PCR-based techniques are highly sensitive and specific experimental tests for detecting trypanosomal DNA in either the vector or the host. PCR has been shown to be useful for the detection of trypanosome infections in cattle with haematocrit values below 25 % (Solano *et al.* 1999). Nevertheless, PCR has been scarcely used to assess the prevalence of trypanosomosis in field samples due to its time-consuming, prohibitive cost and requirement for technical expertise (Solano *et al.* 1999). Furthermore, PCR requires specialised equipment and is not suitable for use as a basis for treatment of individual animals, especially in remote rural areas.

Despite the availability of the above parasitological, serological and molecular tests for the diagnosis of animal trypanosomosis, there are no simple pen-side diagnostic tests for 'on-spot' diagnosis of individual cases of animal trypanosomosis. Under such circumstances field veterinarians must make a clinical diagnosis before treating cases. The common clinical signs of animal trypanosomosis include anaemia, fever, staring coat, enlarged superficial lymph nodes, wasting, petechial haemorrhages in and pallor of mucous membranes, ocular discharge, diarrhoea and abortion (Stephen 1986). The sensitivity of a clinical diagnosis based on these signs is not known. In this study the efficiency of a clinical diagnosis was compared to that of parasitological and molecular diagnoses.

MATERIALS AND METHODS

Study area

This work was conducted in Tororo and Soroti districts located in Eastern Uganda. The vegetation and climate of the study area have been described in detail by Magona & Mayende (2002).

Sample collection and analysis

Two hundred and fifty cattle were bled from the jugular vein using a vacutainer and about 10 mℓ of blood were taken from each animal. Some blood was used for parasitological examination for trypanosomes using both the haematocrit centrifugation technique and the buffy coat technique. The remainder of the blood samples were centrifuged at 1 000 *g* for 5 min and the buffy coat was decanted and kept under liquid nitrogen until the extraction of trypanosome DNA and PCR amplification was carried out using primers specific for *Trypanosoma brucei*, *T. vivax*, *T. congolense* Savannah-, Kilifi-, Tsavo- and forest-types as described by Kimmel, Ole-MoiYoi & Young (1987); Majiwa & Otieno (1990); Masiga, Smyth, Hayes, Bromidge & Gibson (1992); Majiwa, Maina, Waitumba, Mihok & Zweygarth (1993) and Masake, Majiwa, Moloo, Makau, Njuguna, Kabata, Ole-MoiYoi & Nantulya (1997). Packed cell volume (PCV) was measured using PCV reader (Hawksley, England). Haemoglobin (Hb) concentration was measured using the WHO Haemoglobin Colour Scale (Stott & Lewis 1995).

Clinical examination

A general physical examination was conducted on all cattle bled. The prescapular and prefemoral lymph nodes were palpated to determine whether they were enlarged. The skin coat was examined for any sign of roughness or staring. Ocular and vulva mucous membranes were examined for presence of pallour or petechial haemorrhages, and the presence of diarrhoea, and ocular and/or nasal discharges was noted.

The body condition was assessed visually based on a nine score system of F+, F, F–; M+, M, M–; L+, L and L–, in which L– represents 1 and F+ represents 9 (Nicholson & Butterworth 1986). F stands for fat, M for medium and L for lean. Rectal temperature was taken on each animal and those with a temperature of > 39.4 °C were considered to be pyrexic. PCV and Hb concentration were measured to detect anaemia. Animals with a PCV of < 25 % or Hb concentration of < 8 g/dℓ were considered anaemic. Any history of abortions or stillbirths in the herds was obtained from the cattle owners.

RESULTS

Of the 250 cattle examined, the clinical, molecular (PCR assay) and parasitological diagnoses detected 184 (73.6 %), 96 (38.4 %) and 36 (14.4 %) of the

animals respectively as diseased or harbouring trypanosome infections.

In Tables 1 and 2 the sensitivity and specificity of the clinical examinations, based on parasitological and molecular tests as the goldstandards, is given. The clinical examinations had a good sensitivity (78%), but a low specificity (27%) when compared to parasitological tests (Table 1). In relationship to PCR, clinical examinations had a high sensitivity (87.5%) and a low specificity (35%) (Table 2).

In Table 3 the results of the detection rate of clinical examination for trypanosome infections caused by different trypanosome species which were confirmed by parasitological tests, are given. Clinical examination had an excellent detection rate (100%) for mixed infections of *T. brucei* with *T. congolense,* and *T. brucei* with *T. vivax.* It also had a good detection rate for single *T. vivax* infections (84%) and single *T. brucei* infections (78%), but only one out of three single *T. congolense* infections were diagnosed clinically.

TABLE 1 Sensitivity and specificity of clinical examination for natural trypanosome infections in cattle in Uganda when parasitological tests are used as the gold standard

Clinical examination	Parasitological diagnosis		Total
	Trypanosomosis-infected	Trypanosomosis-free	
Positive	28	156	184
Negative	8	58	66
Total	36	214	250

Sensitivity = 28/36 x 100 = 78 %
Specificity = 58/214 x 100 = 27 %

TABLE 2 Sensitivity and specificity of clinical examination for natural trypanosome infections in cattle in Uganda when PCR is used as the gold standard

Clinical examination	Molecular diagnosis		Total
	Trypanosomosis-infected	Trypanosomosis-free	
Positive	84	100	184
Negative	12	54	66
Total	96	154	250

Sensitivity = 84/96 x 100 = 87.5 %
Specificity = 54/154 x 100 = 35 %

TABLE 3 The detection rate of clinical examination for parasitologically confirmed trypanosome infections of cattle in Uganda

Type of trypanosome infection	No. confirmed by parasitological methods	No. detected by clinical diagnosis	Detection rate of clinical diagnosis (%)
T. brucei	33	26	78
T. congolense	3	1	33
T. vivax	13	11	84
T.v/T.c***	1	0	0
*T.b***/T.c*	1	1	100
T.b/T.v	9	9	100
T.b/T.c /T.v	1	0	0
Total	36	28	78

* *T.v = Trypanosoma vivax*
** *T.c = Trypanosoma congolense*
*** *T.b = Trypanosoma brucei*

TABLE 4 The detection rate of clinical examination for PCR-confirmed trypanosome infections in cattle in Uganda

Type of trypanosome infection	No. confirmed by PCR	No. detected by clinical examination	Detection rate of clinical examination (%)
T. brucei	89	74	83
T. vivax	12	11	91
T.c-forest**	3	3	100
T.c-Savannah	6	4	67
T.c-Tsavo	27	22	81
T.b/Savannah/Tsavo	3	1	33
T.b/*T.v**	8	7	87
T.c-Tsavo/*T.v*	1	1	100
T.c-Tsavo/*T.b****	10	7	70
T.b/*T.c*-forest/*T.c*-Tsavo	1	1	100
T.b/*T.v*/*T.c*-Tsavo	1	1	100
Total	96	84	87

* *T.v* = *Trypanosoma vivax*
** *T.c* = *Trypanosoma congolense*
*** *T.b* = *Trypanosoma brucei*

Table 4 shows the detection rate of clinical examinations for trypanosome infections caused by different trypanosome species as confirmed by molecular diagnosis. Clinical examination detected all infections due to *T. congolense* forest-type (3), mixed infections of *T. congolense* Tsavo-type with *T. vivax* (1), mixed infections of *T. congolense* forest- and Tsavo–type with *T. brucei* (1), and mixed infections of *T. congolense* Tsavo-type with *T. brucei* and *T. vivax* (1). The detection rate of clinical examinations for single infections due to *T. vivax* (91 %), *T. brucei* (83 %) and *T. congolense* Tsavo-type (81 %) and mixed infections of *T. brucei* with *T. vivax* (87 %) was good, but it was fair for mixed infections of *T. congolense* Tsavo-type with *T. brucei* infections (70 %) and *T. congolense* Savannah-type (67 %).

Both parasitological and molecular techniques revealed *T. brucei* to be the predominant trypanosome infection, followed by *T. vivax* and *T. congolense*. Molecular diagnosis further revealed *T. congolense* Tsavo-type (17.2 %) to be more prevalent than *T. congolense* Savannah-type (3.6 %) and forest-type (1.6 %). No infections of *T. congolense* Kilifi-type were detected.

DISCUSSION

In this study the efficiency of the clinical diagnosis of bovine trypanosomosis was compared to that of parasitological and molecular diagnoses in cattle suffering from natural trypanosome infections in Uganda. In comparison to the parasitological diagnosis, clinical examination had a good sensitivity, but a low specificity, and when compared to the molecular diagnosis, it had a high sensitivity, but a poor specificity. The good sensitivity displayed by clinical examination means that many cases of trypanosome infections manifest clinical signs, the basis for clinical diagnosis, and it could be a useful means for the detection and treatment of cases of bovine trypanosomosis. However, many clinical signs of animal trypanosomosis are similar to those of other parasitic infections, such as theileriosis, anaplasmosis, babesiosis, fasciolosis and gastrointestinal nematode infections (Molyneux & Ashford 1983). Thus many false positive diagnoses based on clinical examination grounds may be made which diminishes its specificity.

Clinical examination had the highest detection rate for mixed infections of *T. brucei* with *T. congolense*, or *T. brucei* with *T. vivax*. For single trypanosome infections, clinical examination had a high detection rate for *T. vivax* and *T. brucei* infections but a low detection rate for *T. congolense* infections. This implies that the majority of cattle suffering from mixed trypanosome infections manifest clinical signs. For single trypanosome infections, more *T. vivax* and *T. brucei* infections were associated with clinical signs than *T. congolense* infections. This could be attributed to fact that the number of *T. congolense* infections, revealed by parasitological tests, was not sufficient to arrive at a meaningful conclusion, because *T. congolense* infections were the least prevalent as compared to those of *T. brucei* and *T. vivax* infections. On the other hand, this could have been due to the fact that less pathogenic trypanosome species, such as *T. brucei* and *T. vivax*,

tend to cause more chronic long-lasting infections, which are often more associated with clinical manifestation than the more pathogenic trypanosome species, such as *T. congolense,* that cause an acute, fatal disease. This must, however, be interpreted with caution because the diagnosis of many single and mixed infections are missed when parasitological techniques are used. Direct microscopy, in general, is thought to miss 50% (Barnett 1947).

Most of the trypanosome infections caused by single or mixed infections of *T. congolense* forest- and Tsavo-types with either *T. vivax* or *T. brucei* were detected by clinical examination. It was evident that more single or mixed infections of *T. congolense* forest- and Tsavo-types were associated with clinical manifestation than those of *T. congolense* Savannah-type. This probably implies that *T. congolense* forest-and Tsavo-type are less pathogenic than *T. congolense* Savannah-type in cattle and are associated with a more chronic long-lasting syndrome that manifests clinically. Evidence from experimental infections of cattle with Savannah-, forest- and Kilifi- *T. congolense*-types confirmed that Savannah-type is more pathogenic and causes an acute and fatal disease with animals dying within 29–54 days (Sidibe, Bengaly, Boly, Ganaba, Desquesnes & Sawadogo 2002). Whereas the forest- and Kilifi-types cause a less severe infection that induces the development of clinical signs such as pyrexia, anaemia, listlessness, loss of appetite and staring hair coat, these signs disappear as the disease progresses and ultimately animals recover without treatment about 3 months after infection (Sidibe *et al.* 2002). The findings of this study are consistent with the suspicion that differences in pathogenicity of different morphological (Godfrey 1960; 1961) and genotypic forms (Majiwa, Masake, Nantulya, Hammers, Van Meirvenne & Matthyssens 1985; Majiwa, Hammers, Van Meirvenne & Matthyssens 1986) of *Trypanosoma congolense* result in different trypanosomosis syndromes.

The finding that *T. brucei* infections in the cattle were more predominant than *T. vivax* and *T. congolense* infections in the study area, which apparently was sleeping sickness endemic, is consistent with the previous finding that in south-eastern Uganda, *T. brucei* infection is predominant in domestic animals in sleeping sickness endemic areas, while *T. vivax* is predominant in areas outside sleeping sickness areas (Magona, Kakaire & Mayende 1999). Molecular diagnosis revealed that *T. congolense* Tsavo-type was more predominant than *T. congolense* Savannah-type and forest-type contrary to reports by Mugittu, Silayo, Majiwa, Kimbita, Mutayoba & Maselle (2001) on farms in the Morogoro region of Tanzania, where *T. congolense* Savannah- and Kilifi-types were detected and the Savannah-type was predominant. These differences in the distribution of the different *T. congolense* genotypes could be related to the species of tsetse vector involved in the area under question. Whereas in south-eastern Uganda it is the Riverine group of tsetse (*Glossina fuscipes fuscipes*) that is the most important transmitter of bovine trypanosomosis, it is the *morsitans* group of tsetse involved in the area reported on by Mugittu *et al.* (2001).

Clinical diagnosis seems to be useful in detection of chronic trypanosome infections in cattle, which are often associated with clinical signs and exert an immense impact on cattle productivity. Many of the infections seem to be due to *T. congolense* forest- and Tsavo-type and their mixed infections with *T. vivax* and *T. brucei* in Uganda. Chronic *T. vivax* infections are usually less severe than *T. congolense* infections and their mixed infections with *T. vivax* and *T. brucei.* They manifest as progressive anaemia, staring hair coat, emaciation, swelling of prescapular and prefemoral lymph nodes, abortion and decrease in milk production (Stephen 1986). This form of trypanosomosis is in some cases associated with severe wasting of animals, hence the name ' thin cow syndrome' that is given to it on the Kenya coast (Dowler, Schillinger & Connor 1989). Given that clinical diagnosis can detect a large proportion of chronic trypanosome infections, the stage of infection often missed by parasitological diagnosis, and that the latter do detect the majority of acute or early stage trypanosome infections in which parasitaemia is generally reasonably high, a combination of thorough clinical examination of cases and parasitological diagnosis would be very useful for field veterinarians to employ in the control of bovine trypanosomosis in remote areas of Africa.

In conclusion, under field conditions, in the absence of simple and portable diagnostic tools or access to laboratory facilities, veterinarians could rely on clinical diagnosis to screen and treat cases of bovine trypanosomosis presented by farmers before confirmatory diagnosis in diagnostic centres for few unclear cases is sought.

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REFERENCES

BARNETT, S.F. 1947. Bovine trypanosomiasis in Kenya with special reference to its treatment with phenanthridium. *Veterinary Record,* 59:459–462.

DESQUESNES, M.1996. Evaluation of three antigen detection tests (monoclonal trapping ELISA) for African trypanosomes with an isolate of *Trypanosoma vivax* from French Guyana. *Annals of New York Academy of Sciences*, 791:172–184.

DOWLER, M.E., SCHILLINGER, D. & CONNOR, R.J. 1989. Notes on the routine intravenous use of isometamidium in the control of bovine trypanosomiasis on the Kenya coast. *Tropical Animal Health and Production*, 21:4–10.

EISLER, M.C., LESSARD, P., MASAKE, R.A. MOLOO, S.K. & PEREGRINE, A.S. 1998. Sensitivity and specificity of antigen-capture ELISAs for diagnosis of *Trypanosoma congolense* and *Trypanosoma vivax* infections in cattle. *Veterinary Parasitology*, 79:187–201.

GODFREY, D.G. 1960. Types of *Trypanosoma congolense*. I: Morphological differences. *Annals of Tropical Medicine and Parasitology*, 54:428–438.

GODFREY, D.G. 1961. Types of *Trypanosoma congolense* II: Difference in the course of infections. *Annals of Tropical Medicine and Parasitology*, 55:154–166.

HOPKINS, J.S., CHITAMBO, H., MACHILA, N., LUCKINS, A.G., RAE, P.F., VAN DEN BOSSCHE, P. & EISLER, M.C. 1998. Adaptation and validation of antibody-ELISA using dried blood spots on filter paper for epidemiological surveys of tsetse-transmitted trypanosomosis in cattle. *Preventive Veterinary Medicine*, 37:91–99.

KIMMEL, B.E., OLE-MOIYOI, O.K. & YOUNG, J.R. 1987. Ingi, a 5.2 Kb dispersed element from *Trypanosoma brucei* that carries half of a smaller mobile element at either end and has homology with mammalian lines. *Molecular Cellular Biology*, 7:1465–1475.

LUCKINS, A.G. 1977. Detection of antibodies in trypanosome-infected cattle by means of a microplate enzyme-linked immunoassay. *Tropical Animal Health and Production*, 9: 53–62.

MAGONA, J.W., KAKAIRE, D.W. & MAYENDE, J.S.P. 1999. Prevalence and distribution of animal trypanosomosis on Buvuma Islands in Lake Victoria, Uganda (Short Communication). *Tropical Animal Health and Production*, 31:83–87.

MAGONA, J.W. & MAYENDE, J.S.P. 2002. Occurrence of concurrent trypanosomosis, theileriosis, anaplasmosis and helminthosis in Friesian, Zebu and Sahiwal cattle in Uganda. *Onderstepoort Journal of Veterinary Research*, 69:133–140.

MAJIWA, P.A.O., MASAKE, R.A., NANTULYA, V.M., HAMMERS, R., VAN MEIRVENNE, N. & MATTHYSSENS, G. 1985. *Trypanosoma (Nannomonas) congolense*: Identification of two karyotype groups. *EMBO Journal*, 12:3301–3313.

MAJIWA, P.A.O., HAMMERS, R., VAN MEIRVENNE, N. & MATTHYSSENS, G. 1986. Evidence of genetic diversity in *Trypanosoma (Nannomonas) congolense. Parasitology*, 93: 291–304.

MAJIWA, P.A.O. & OTIENO, L.H. 1990. Recombinant DNA probes reveal simultaneous infection of tsetse flies with different trypanosome species. *Molecular Biochemistry and Parasitology*, 40:245–254.

MAJIWA, P.A.O, MAINA, M., WAITUMBA, J.N., MIHOK, S. & ZWEYGARTH, E. 1993. *Trypanosoma (Nannomonas) congolense*: molecular characterization of a new genotype from Tsavo, Kenya. *Parasitology*, 106:151–152.

MASAKE, R.A., MAJIWA, P.A.O., MOLOO, S.K., MAKAU, J.M., NJUGUNA, J.T., KABATA, J., OLE-MOIYOI, O.K. & NANTULYA, V.M. 1997. Sensitive and specific detection of *T. vivax* using polymerase chain reaction. *Experimental Parasitology*, 85:193–205.

MASIGA, D.K., SMYTH, A.J., HAYES, P., BROMIDGE, T.J. & GIBSON, W.C. 1992. Sensitive detection of trypanosomes in tsetse flies by DNA amplification. *International Journal of Parasitology*, 22:909–918.

MOLYNEUX, P.H. & ASHFORD, P.W. 1983. *The biology of Trypanosoma and Leishmania, Parasites of man and domestic animals.* London: Taylor & Francis.

MUGITTU, K.N., SILAYO, R.S., MAJIWA, P.A.O., KIMBITA, E.K., MUTAYOBA, B.M. & MASELLE, R. 2001. Application of PCR and DNA probes in the characterisation of trypanosomes in the blood of cattle in farms in Morogoro, Tanzania. *Veterinary Parasitology*, 94:177–189.

MURRAY, M., MURRAY, P.K. & MCINTYRE, W.I.M. 1977. An improved parasitological technique for the diagnosis of African trypanosomiasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 71:325–326.

NANTULYA, V.M. & LINDQVIST, K.J. 1989. Antigen-detection enzyme immunoassays for the diagnosis of *Trypanosoma vivax*, *T. congolense* and *T. brucei* infections in cattle. *Tropical Medicine and Parasitology*, 40:267–272.

NICHOLSON, M.J. & BUTTERWORTH, M.H. 1986. *A guide to condition scoring of zebu cattle.* Addis Ababa: International Livestock Centre for Africa (ILCA).

REBESKI, D.E., WINGER, E.M., ROGOVIC, B., ROBINSON, M.M., CROWTHER, J.R. & DWINGER, R.H. 1999. Improved methods for the diagnosis of African trypanosomosis. *Memorias do Instituto Oswald Cruz*, 94:249–253.

SIDIBE, I., BENGALY, Z., BOLY, H., GANABA, R., DESQUESNES, M. & SAWADOGO, L. 2002. Differential pathogenicity of *Trypanosoma congolense* subgroups: Implications for the strategic control of trypanosomosis. *Newsletter on Integrated Control of Pathogenic Trypanosomes and their Vectors*, No. 6, September 2002: 33–35.

SOLANO, P., MICHEL, J.F., LEFRANCOIS, T., DE LA ROCQUE, S., SIDIBE, I., ZOUNGRANA, A. & CUISANCE, D. 1999. Polymerase chain reaction as a diagnosis tool for detecting trypanosomes in naturally infected cattle in Burkina Faso. *Veterinary Parasitology*, 86:95–103.

STEPHEN, L.E. 1986. *Trypanosomiasis: a veterinary perspective.* Oxford: Pergamon Press.

STOTT, G.J. & LEWIS, S.M. 1995. A simple and reliable method for estimating haemoglobin. *Bulletin of World Health Organization*, 73:369–373.

WOO, P.K.T. 1969. The haematocrit centrifugation technique for the detection of trypanosomiasis in blood. *Canadian Journal of Zoology*, 47:921–923.