J. Protozool. Res. 17, 57-63 (2007)
Copyright©2007, National Research Center for Protozoan Diseases

The application of a combination of diagnostic tests for bovine trypanosomosis is vital for an effective control of the disease

Mbwambo, H.A.*, Ngovi, C.J. and Meela, E.S.
Central Veterinary Laboratory Temeke, P.O. Box 9254, Dar Es Salaam, Tanzania
*Corresponding author: Heriel A. Mbwambo, E-mail: herimbwambo@yahoo.com

ABSTRACT

Four hundred thirty two cattle in 9 farms in the Coast and Dar Es Salaam regions of Tanzania were bled at different periods from March 2006 to January 2007 and specimens prepared for parasitological (buffy coat technique - BCT, blood slide examination) and serological (Antigen, Antibody, PCR ELISAs and LAMP Test) screening for presence of trypanosomes. Alongside this, 1.0 ml of EDTA blood of test animals with packed cell volume (PCV) values less than 25 was inoculated into 20 g mouse for possible detection of trypanosomes in low parasitaemic infections; were followed-up for 60 days. Four cattle were detected positive for pathogenic trypanosomes, notably *Trypanosoma vivax* in three cattle by blood slide examination and BCT, and *T. congolense* (one case) by mouse inoculation. Serological screening is not yet completed. 90.3% of animals sampled had PCV values above 25, while 42 cattle (9.7%) had PCV values below 25. Low PCV values in adult cattle in trypanosomosis-endemic areas suggest presence of trypanosome infections. Subsequent trypanocidal drug treatment of five parasitologically negative cattle with PCV of 15, 16, 18, 20 and 22 resulted into improved PCV values of ≥25 ≤27 by Day 30 after treatment with diminazene aceturate 7.0mg per kg body weight. Mouse inoculation was discriminately done on blood of parasitologically negative cattle with PCV values below 25. One pair of mice became positive for *T. congolense* on Day 43 and 47 of inoculation. Although results from more sensitive diagnostic tests are not yet available, the individual parasitological tests (blood slide, BCT, mouse inoculation) and trypanocidal drug treatment of low PCV-BCT-negative adult cattle have shown complementarities to one another; each option has place in perfecting trypanosomosis diagnosis for an improved control of the disease.

Key words: bovine-trypanosomosis, buffy-coat-technique, packed-cell-volume, mouse-inoculation, trypanocidal-drug-treatment, serological tests

INTRODUCTION

Trypanosomosis continues to be one of the most important disease conditions in Tanzania affecting not only livestock, but also man. About 4.4 million cattle are at risk of contracting Nagana and about 4.0 million people are at risk of contracting human sleeping sickness. It is the tsetse fly of the Genus *Glossina* that has for decades maintained effective cyclical transmission of the pathogenic trypanosomes. There are seven major species of tsetse in Tanzania, namely: *Glossina morsitans, G. swynnertoni, G. austeni, G. pallidipes, G. fuscipes, G. palpalis* and *G. brevipalpis.* Control of tsetse and trypanosomosis is highly essential in order to avoid or minimize losses in productivity either directly as a result of mortality and morbidity or indirectly through its impact on land use and rural development. The fight against trypanosomosis is twofold, directed on the vector tsetse and the diseases transmitted, which unfortunately is constrained by not only the high costs of control, but also drug resistance (Geerts and Holmes, 1998; Mbwambo *et al.*, 1988; Mbwambo *et al.*, 2001). Furthermore, the economies of most developing countries of Africa where tsetse and trypanosomosis are endemic are very low. Before any effective control operation of

trypanosomosis is implemented there should be a thorough assessment of disease situation, which requires the availability of pen side diagnostic tests that are affordable and practicable in the affected countries/areas. And, since the available diagnostic techniques for trypanosomosis diagnosis, surveillance and control have failed to provide an effective control of the disease when used in isolation, integration of improved techniques in disease diagnosis with vector and disease control methods, remain a more suitable alternative for a sustained disease control strategy. The antigen and antibody-detection ELISAs for trypanosomosis diagnosis are established at the Central Veterinary Laboratory Temeke by Mbwambo, 1997 and Mbwambo *et al.*, 2000, respectively, but are not used on routine basis. It is for the sole purpose of integrating novel improved techniques into trypanosomosis control strategies, that the LAMP (Loop-mediated isothermal amplification of DNA) is being validated in several laboratories including the Central Veterinary Laboratory, Temeke Dar Es Salaam Tanzania, for possible inclusion of the technology as a complementary tool to the available, but less sensitive diagnostic tests.

MATERIALS AND METHODS

Cattle and samples

Four hundred thirty two cattle in 9 farms in the Coast and Dar Es Salaam regions of Tanzania were bled, at different periods from March 2006 to January 2007, from the marginal ear vein for preparation of blood smears, buffy coats and dried blood on FTA® Classic Card, Whatman®. Alongside this, jugular vein blood was collected into plain and EDTA vacutainer tubes, for preparation of sera, and mouse inoculation, respectively.

Diagnostic Techniques

Parasitological diagnosis

Blood smears were air-dried, fixed in methanol (thin blood smears only), stained with Giemsa's stain for 45 minutes and examined under microscope for presence of trypanosomes. Blood was collected into heparinized capillary tubes, centrifuged at 12,000 rpm for 5 minutes and the buffy coat examined for trypanosomes using the dark ground phase-contrast buffy-coat technique (DG BCT) as described by Murray *et al.*, 1977. Alongside this, 1.0 ml of EDTA blood of test animals with packed cell volume (PCV) values less than 25 was inoculated into app. 20 g mouse for possible detection of trypanosomes in low parasitaemic infections; were followed-up by microscopic examination of wet mount of tail tip blood under cover slip, on alternate days for 60 days. Five parasitologically-negative cattle with PCV values below 23 were given intramuscular injection of diminazene aceturate 7.0 mg per kg body weight.

Serological diagnosis

Serum samples were stored at -20°C, and dried blood on FTA cards stored at room temperature, for screening for presence of trypanosomal antibodies, trypanosomal antigen by enzyme-linked immunosorbent assay, and trypanosomal DNA by polymerase chain reaction and loop-mediated isothermal amplification (LAMP). Serological and parasitological data of samples previously collected by the authors from Mafia Island of Coast region of Tanzania were used to elucidate the complementarities of the individual diagnostic tests to one another.

RESULTS

Parasitological diagnosis

Examination of Giemsa-stained blood slides, DG BCT and mouse inoculation

Three cattle were detected positive for *T. vivax* by blood slide and BCT technique, and one cow (0.9%) positive for *T. congolense* by mouse inoculation (one pair) on Day 43 and 47 of inoculation, respectively (Table 1). Blood of 114/432 (26.4%) cattle was inoculated into mice. 90.3% of animals sampled had PCV values above 25, while 42 cattle (9.7%) had PCV values below 25 with a range of 15 – 50 (Table 1 and Table 2). Neither *T. brucei, Anaplasma, Babesia, Theileria* nor other blood parasites were detected.

Trypanocidal drug intervention of BCT-negative-low PCV- cattle

Subsequent trypanocidal drug treatment of five parasitologically negative cattle with PCV values of 15, 16, 18, 20 and 22 resulted into improved PCV values of ≥25 ≤27 by Day 30 after treatment with diminazene aceturate 7.0 mg per kg body weight.

Serological diagnosis

Current samples: Work not yet completed.

Mafia Island samples

Parasitological and serological results of 12-*T. congolense*-positive samples collected on Mafia Island of the Coast region, Tanzania, are illustrated on Table 3, on which four (5) cattle were positive by BCT only, one by mouse inoculation and examination of tail tip wet mount, five (5) by PCR only and one by both BCT and PCR techniques. Also, among other 15 *T. theileri*-positive samples, 6 were found positive by BCT only, four (4) by PCR and five (5) by both techniques (Table 4).

DISCUSSION

Serological tests for trypanosomosis diagnosis were developed in early seventies, however, were reported to be less definitive than parasitological tests when used alone and were not completely reliable for the diagnosis of trypanosomosis in individual animals (Wilson and Cunningham, 1971; Ashkar and Ochilo, 1972; Zwart *et al*., 1973). The Antibody detection ELISA developed by Luckins A.G. 1977 showed improved sensitivity, however, lacked species specificity and could not distinguish current infection from a previous one. The development of antigen-detection ELISA (Nantulya *et al*., 1987) was expected to overcome the shortfalls of Ab-ELISA, however, its sensitivity and specificity were later discovered to be unsatisfactory (Eisler *et al*., 1997). Nevertheless, the Ag-detection ELISA was indeed useful on Unguja Island of Zanzibar. Mangapwani area of Unguja Island, previously reported to be free of tsetse and trypanosomosis based on parasitological results alone, was found to be antigenaemic (was not free from the disease) following application of the Ag-detection ELISA (Mbwambo and Mpokwa, 1993), and control operation had to start afresh. DNA-based (Polymerase Chain Reaction–PCR- ELISAs (Moser *et al*., 1989; Weiss, 1995; Masake *et al*., 1997; Almeida *et al*., 1997), and the Loop-mediated isothermal amplification (LAMP) of trypanosomal DNA, Kuboki *et al*., 2003) have even more improved sensitivity and specificity. Thus the advancement of science and technology has lead to the establishment of diagnostic tests with increased sensitivity and specificity. However, tests with improved sensitivity and specificity are complex and require the use of expensive equipment and expertise.

Table 1: Preliminary results of 432 cattle screened for presence of trypanosomes from 8 dairy farms and one beef ranch on Mainland Tanzania, in the year 2006/2007.

Name of Farm	Total No. Cattle	PCV (Av)	PCV Range	BS/ BCT	Mouse Inoculation	Serology
Alidina Farm	24	26.3	18 - 34	-ve	10/24 –ve; D60	NYD
Kiguza Farm	92	29.8	15 - 50	2*T. v*[1]	19/92, 1*Tc*[2]; D60	NYD
Mgawa Farm	12	31.2	25 - 41	1*T. v*[1]	5/12 –ve; D60	NYD
Zico Farms	80	30.4	18 - 40	-ve	18/80 –ve; D60	NYD
Bakiga Farm	44	32.7	20 - 42	-ve	19/44–ve; D60	NYD
NARCO Ruvu	48	33.4	16 - 48	-ve	8/48 –ve; D60	NYD
Dimara Farm	36	32.1	20 - 42	-ve	12/36 –ve; D60	NYD
Kwambota Farm	48	31.0	20 - 46	-ve	11/48 –ve; D60	NYD
LMU Kibaha	48	33.2	18 - 45	-ve	12/48 –ve; D60	NYD

PCV = packed cell volume; Av = Average; -ve = No parasites seen; BS/BCT = Blood slide examination and/or buffy coat technique; D60 = Duration of observation period is 60 days; Tv[1] = *Trypanosoma vivax*; Tc[2] **=** *Trypanosoma congolense* on Day 43 of mouse inoculation**;** LMU = Livestock multiplication unit.

Table 2. Average packed cell volume values of 432 cattle from 8 dairy farms and one beef ranch on Mainland Tanzania, in the year 2006/7.

Name of Farm	Proportion (%) of Cattle with Packed Cell Volume (PCV)			
	≥30	≥27<30	≥25<27	≥15<25
Alidina Farm	8/24 (33.3)	4/24 (16.7)	4/24 (16.7)	8/24 (33.3)
Kiguza Farm	52/92 (56.5)	12/92 (13.0)	17/92 (18.5)	11/92 (12.0)
Zico Farms	49/80 (61.3)	18/80 (22.5)	7/80 (8.7)	6/80 (7.5)
Bakiga Farm	37/44 (84.0)	5/44 (11.4)	1/44 (2.3)	1/44 (2.3)
Mgawa Farm	8/12 (66.7)	1/12 (8.3)	3/12 (25.0)	0 (0)
Dimara Farm	29/36 (80.5)	2/36 (5.6)	2/36 (5.6)	3/36 (8.3)
Kwambota Farm	29/48 (60.4)	6/48 (12.5)	7/48 (14.6)	6/48 (12.5)
LMU Kibaha	37/48 (77.1)	4/48 (8.3)	1/48 (2.1)	6/48 (12.5)
NARCO Ruvu	41/48 (85.4)	4/48 (8.3)	2/48 (4.2)	1/48 (2.1)
Total	290/432(67.1)	56/432(13.0)	44/432 (10.2)	42/432 (9.7)
		390/432 (90.3)		42/432 (9.7)

LMU = Livestock Multiplication Unit; NARCO = National Ranching Company.

The choice of a diagnostic kit for any test would ideally depend on the purpose for which the test is required, although it may also be influenced by the amount of money that would be available for the purpose. To the poor farmer, any meaningful diagnostic result remains the one that can allow effect appropriate control measure as early as is possible in order to minimize losses that could result due to death(s) or reduced productivity in delayed or inappropriately attended cases. And, this is only possible if pen side diagnostic tests are available and affordable. However sensitive and specific a test may be, to an ordinary livestock keeper with limited resources, and for the purpose of trypanosomosis control, parasitological techniques give fairly quick results and are comparatively far less expensive.

Trypanosomosis is characterized by protracted fluctuating parasitaemias, with decreased PCV values (anaemia). The ability of trypanosome-infected cattle to control development of anaemia is a criterion of trypanotolerance; is measured by PCV and is most closely linked to cover overall cow productivity with the other possible causes of anaemia controlled (Trail *et al*., 1991). Since animals that cannot maintain PCV values when infected exhibit poor growth (Trail *et al*., 1992), follow-up of the tendency of PCV values in trypanosomosis endemic areas has allowed establishing a threshold, whereby, trypanocidal drug intervention in cattle with decreasing PCV values resulted into improvement of PCV and productivity (Mbwambo *et al*., 2003). During the current studies, use of the advantage of the DG BCT technique, which offers for the examination of buffy coats and determination of the degree of anaemia (PCV), and intervention by administration of the trypanocidal drug diminazene aceturate to blood-slide-BCT-negative cattle with decreased PCV values helped control the disease in affected cattle, thus further confirming the previous finding. Although tedious, xenodiagnosis may also help reveal trypanosome infection in blood-slide-BCT-negative cattle with decreased PCV values. It is apparent from the present studies, that despite the high proportion of mouse inoculation 114/432 (26.4%) only one animal was positive for *T. congolense* (Table 1). Indeed not all *T. congolense* strains do propagate in mice. None of the *T. vivax* detected could propagate in mice also. Use of other hosts like sheep and calves could have revealed more information.

Table 3. Results of 12-*Trypanosoma congolense*-positive cattle of Mafia Island Tanzania, as detected by a combination of diagnostic techniques.

	Parasitological & Serological Techniques		Number Positive by Individual Techniques		
Trypanosome species	Techniques Combined/ Alone	Number Positive	DG BCT	Wet Mount	PCR
Trypanosoma congolense	DG BCT+ve; PCR+ve	1[1]	1[1]	NA	1[1]
	DG BCT+ve only	5	5	NA	0[2]
	Mouse inoculation only	1	0	1	0[2]
	DG BCT-ve; PCR+ve	5	0	NA	5
Total		12	6	1	6[1]

DG BCT = Dark ground buffy coat technique; PCR = Polymerase chain reaction (Conducted at The Inst. for Trop. Vet. Med., Antwerp Belgium); +ve = Positive; -ve = Negative; [1] = One animal positive for *Trypanosoma congolense* by both DG BCT and PCR techniques; [2] = PCR not done.

Table 4. Results of 15-*Trypanosoma theileri*-positive cattle of Mafia Island Tanzania, as detected by a combination of diagnostic techniques.

	Parasitological & Serological Techniques		Number Positive by Individual Techniques	
Trypanosome Species	Techniques Combined /Alone	Number Positive	DG/BCT	PCR
Trypanosoma theileri	DG BCT+ve; PCR+ve	5[1]	5[1]	5[1]
	DG BCT+ve; PCR-ve	3	3	0
	DG BCT+ve; PCR ND	3	3	0
	DG BCT-ve; PCR+ve	4	0	4
Total		15	11	9

DG BCT = Dark ground buffy coat technique; PCR = Polymerase chain reaction (Conducted at The Inst. for Trop. Vet. Med., Antwerp Belgium); +ve = Positive; -ve = Negative; ND = Not done; [1] = Five animals positive for *Trypanosoma theileri* by both DG BCT and PCR techniques.

Serological tests are not yet done, however, the available parasitological results and corresponding data (parasitological and serological) on samples collected by the authors on Mafia Island of Coast region Tanzania, give enough information to support the complementarities of the diagnostic tests for animal trypanosomosis. The use of PCR revealed five cases of *T. congolense*, which were not detected by the DG BCT technique. On the other hand, six cases would have been missed if mouse inoculation (one case) and DGBCT techniques (five cases) were not used (Table 3). Hence, each diagnostic test has its place in perfecting trypanosomosis diagnosis. And, where advanced techniques are not feasible, parasitological techniques are very useful for the purpose of trypanosomosis control in individual animals to alleviate poverty.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the support of the Director of CVL Temeke for provision of transport, technical staff of the Protozoology Laboratory of CVL Temeke for their hand in monitoring trypanosome infection in mice, and the National Research Center for Protozoan Diseases of Obihiro University of Agriculture and Veterinary Medicine (OUAVM), Japan, for co-operation and invitation to the 1st International Meeting for Protozoan Diseases at OUAVM.

REFERENCES

Almeida, P.J.L.P de., Ndao, M., Van Meirvenne, N. and Geerts, S. 1997. Diagnostic evaluation of PCR in goats experimentally infected with *Trypanosoma vivax*. Acta Tropica. 66: 45–50.

Ashkar, T. and Ochilo, M. 1972. The application of the indirect fluorescent antibody test to samples of sera and dried blood from cattle in the Lambwe Valley, South Nyanza, Kenya, Bull. W. Hlth Org. 47: 769-772.

Eisler, M.C., Lessard, P., Moloo, S.K., Masake, R.A. and Peregrine, A.S. 1997. Sensitivity and specificity of antigen-capture ELISAs for diagnosis of *Trypanosoma congolense* and *T.vivax* infections in cattle. *In:* Proceedings, International Scientific Council for Trypanosomiasis Research and Control, 24th Meeting, Maputo Mozambique, 1997. OAU/ISCTRC Publ. 119: 179-193.

Kuboki, N., Inoue, N., Sakurai, T., Di Cello, F., Grab, D.J., Suzuki, H., Sugimoto, C. and Igarashi, I. 2003. Loop-mediated isothermal amplification for detection of African trypanosomes. J. C. Microbiol. 41(12): 5517-5524.

Luckins, A.G. 1977. Detection of antibodies in trypanosome-infected cattle by means of a microplate enzyme-linked immunosorbent assay, Trop. Anim. Hlth Prod. 9: 53-62.

Masake, R.A., Majiwa, P.A.O., Moloo, S.K., Makau, J.M., Njuguna, J.T., Maina, M., Kabata, J., Ole-Moi-Yoi, O.K. and Nantulya, V.M. 1997. Sensitive and specific detection of *Trypanosoma vivax* using the polymerase chain reaction. Experimental Parasitology 85: 193–205.

Mbwambo, H.A. 1997. Trypanosomosis surveillance on Zanzibar Island, using the trypanosomal antigen detection ELISA technique. *In:* Proc. of the Workshop on Epidemiological Tools for Monitoring Trypanosomosis and Tsetse Control Programmes IAEA-TECDOC-925 Vienna Austria. pp. 87-93.

Mbwambo, H.A., Mella, P.N.P. and Lekaki, K.A. 1988. Berenil (diminazene aceturate) - resistant *Trypanosoma congolense* in cattle under natural tsetse challenge at Kibaha, Tanzania. Acta Tropica. 45: 239-244.

Mbwambo, H.A. and Mpokwa, M.H. 1993. Antigen detection enzyme-linked immunoassay as an aid to the diagnosis of animal trypanosomiasis in Tanzania. Proc. Improving the diagnosis and control of

trypanosomiasis and other vector-borne diseases of African Livestock, using immunoassay methods. Vienna 1993, IAEA- TECDOC-707 pp. 79-86.

Mbwambo, H.A., Ndung'u, J.M., Murilla, G.A., Munga, L., Sinyangwe, L., Machila, N., Holmes, P.H. and Eisler, M.C. 2001. Trypanocidal Drug Resistance in Tanzania. *In:* Inter. Sci. Council for Trypanosomiasis Research and Control, OAU/ISTRC 2001 pp. 168-174.

Mbwambo, H.A., Ngovi, C.J., Bushiri, P.S. and Mella, E.S. 2000. Validation of Antibody- detection ELISA for use in combination with parasitological tests in monitoring trypanosomosis control programmes. Animal Trypanosomosis: Diagnosis and Epidemiology. FAO/IAEA Co-ordinated Research Programme. International Atomic Energy Agency, Vienna Austria 2000. pp. 165-175.

Mbwambo, H.A., Ngovi, C.J., Meela, E.S. and Daffa, J. 2003. Integration of packed cell volume, parasitological findings and clinical examination of trypanosome-infected cattle in decision making on trypanocidal drug intervention. *In:* Proc., 27th Int. Sci. Council for Trypanosomosis Res. & Control (ISCTRC) Conf., 29th Sept. - 4th Oct., 2003, Pretoria South Africa. ISCTRC Publ. No.122, pp. 444-449.

Moser, D.R., Cook, G.A., Ochs, D.E., Bailey, C.P., McKane, M.R. and Donelson, J.E. 1989. Detection of *Trypanosoma congolense* and *Trypanosoma brucei* sub-species by DNA amplification using the polymerase-catalysed chain reaction. Parasitology 99: 57–66.

Murray, M., Murray, P.K. and McIntyre, W.I.M. 1977. An improved parasitological technique for the diagnosis of African trypanosomiasis. Trans. Roy. Soc. Trop. Med. Hyg. 71: 325–326.

Nantulya, V.M., Musoke, A.J., Rurangirwa, F.R., Saigar, N. and Minja, S.H. 1987. Monoclonal antibodies that distinguish *Trypanosoma congolense, T.vivax* and *T.brucei*. Parasite Immunol. 9: 421-431.

Trail, J.C.M., d'leteren, G.D.M., Feron, A., Kakiese, O., Mulungo, M. and Pelo, M. 1991. Effect of trypanosome infection, control of parasitaemia and control of anaemia development on productivity of N'dama cattle. Acta Trop. 48: 37-45.

Trail, J.C.M., d'leteren, G.D.M., Vivian, P., Yangari, G. and Nantulya, V.M. 1992. Relationship between trypanosome infection measured by antigen detection enzyme immunoassays, anaemia and growth in trypanotolerant N'dama cattle. Vet. Parasitol. 42: 213-223.

Wilson, A.J. and Cunningham, M.P. 1971. Immunological aspects of bovine trypanosomiasis. IV. Patterns in the production of common antibodies,Trop. Anim. Hlth Prod. 3: 133-139.

Zwart, D., Perie, N.M., Keppler, A. and Goedbloed, E. 1973. A comparison of methods for the diagnosis of trypanosomiasis in East African domestic ruminants, Trop. Anim. Hlth. Prod. 5: 79-86.

Weiss, J.B. 1995. DNA probes and PCR for diagnosis of parasitic infections. Clinical Microbiology Reviews 8: 113–130.