RESEARCH COMMUNICATION

Preliminary application and evaluation of loop-mediated isothermal amplification (LAMP) for detection of bovine theileriosis and trypanosomosis in Tanzania

O.M.M. THEKISOE[1], J.D. OMOLO[2], E.S. SWAI[3], K. HAYASHIDA[1, 4], J. ZHANG[1], C. SUGIMOTO[4] and N. INOUE[1]*

ABSTRACT

THEKISOE, O.M.M., OMOLO, J.D., SWAI, E.S., HAYASHIDA, K., ZHANG, J., SUGIMOTO, C. & INOUE, N. 2007. Preliminary application and evaluation of loop-mediated isothermal amplification (LAMP) for detection of bovine theileriosis and trypanosomosis in Tanzania. *Onderstepoort Journal of Veterinary Research*, 74:339–342

The sensitivity of LAMP, PCR and microscopy to detect *Theileria* spp. and *Trypanosoma congolense* in field-derived bovine blood samples from Tanzania was evaluated and compared. No parasites were detected by microscopy. Furthermore, no bovine *Theileria* spp. were detected by LAMP and PCR from all the 24 samples collected from Arusha. Four and one out of 24 samples were positive for *Theileria congolense* infection by LAMP and PCR respectively while, 18 and nine out of 40 samples from Dar es Salaam were positive by LAMP and PCR for *Theileria* spp. Infection, respectively. Although all samples from Dar es Salaam were negative for *Trypanosoma congolense* infections by PCR, 12 out of 40 samples were LAMP positive. Whilst PCR is an established gene amplification method for the detection of *Theileria* and trypanosome parasites, this study introduces LAMP as an alternative molecular diagnostic tool that could be used in large-scale epidemiological surveys.

Keywords: Bovine *Theileria* spp., LAMP, microscopy, PCR, Tanzania *Trypanosoma congolense*

Bovine theileriosis syndromes, which are tick-borne diseases, include East Coast fever (ECF) caused by *Theileria parva*, tropical theileriosis caused by *Theileria annulata* and cerebral theileriosis "Ormilo" caused by *Theileria taurotragi* (Lynen, Loomu, Mlinga, Kessy & Nalitolela 2000). In Tanzania, bovine trypanosomosis, mainly caused by *Trypanosoma congolense* and *T. vivax*, ranks second only to ECF in terms of economic impact on the livestock industry (Mugittu, Silayo, Majiwa, Kimbita, Mutayoba & Maselle 2001). Loop-mediated isothermal amplification (LAMP) is a novel gene amplification method that is rapid, simple and highly sensitive (Notomi, Okayama, Masubuchi, Yonekawa, Watanabe, Amino & Hase 2000). LAMP reaction can be conducted in 60 minutes using a simple laboratory heat block or water bath for incubation and results can be visualized by either gel electrophoresis or by naked eye (white turbidity in reaction tubes) (Mori, Nagamine, Tomita & Notomi 2001). In this study, we evaluated

* Author to whom correspondence is to be directed. E-mail: ircpmi@obihiro.ac.jp

1 National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, 080-8555, Japan

2 Ministry of Livestock Development, Department of Veterinary Services, Box 456, Dodoma, Tanzania

3 Veterinary Investigation Centre, Box 1068, Arusha, Tanzania

4 Research Center for Zoonosis Control, Hokkaido University, Sapporo, Hokkaido, 011-0818, Japan

Accepted for publication 13 March 2007—Editor

and compared the sensitivity of LAMP, PCR and microscopy for the detection of bovine theileriosis and trypanosomosis (*T. congolense*) from cattle samples collected from Tanzania's Arusha and Dar es Salaam farms.

Forty blood samples blotted on filter papers (FTA® card, Whatman, UK) and thin blood smears were prepared from blood collected from cattle of unknown age on ten farms near Dar es Salaam and 24 samples from cattle on seven farms near Arusha (animal age ranged between 4 months and 8 years). Genomic DNA used in LAMP primer specificity and sensitivity tests was extracted according to Sambrook & Russel (2001). The blood samples dried on filter papers were purified according to the manufacturer's instructions and then used as a DNA template. The LAMP primer sets used in the current study were designed from *T. parva* HSP70 (Accession no. U40190) and *T. congolense* 18S rRNA (Accession no. U22315) using Primer Explorer V2 (Fujitsu, Japan), with the following sequences:

LAMP primer set for *T. parva* HSP70

FIP: 5'-TGG GTT ACG GGC TTC TTG GTT TCC TAC GTC GCA TTC ACT GAC-3'; BIP: 5'-ATT TTC GAC GCC AAG AGG CTC AAA TGG CCA GTG CTT CAT GTC-3'; F3: 5'-GGA AAC AGG ACA ACG CCG-3', and B3: 5'-CCG TTT GGT CCG TTG GTA A-3'.

LAMP primer set for *T. congolense* 18S rRNA

FIP: 5'-GCG CAT GCG TCG GTG TTA TTT TCG CGT GTG TGT TCA TGT CA-3'; BIP: 5'-ACT CTC CCC CCA AAA TGG TTG TCC AAG CAC GCA AAT TCA CAT-3'; F3: 5'-TGT GTG TTT GTC GTG GAA GC-3', and B3: 5'-ATT CGT GAC CGC GTC AAA-3'.

Both LAMP and polymerase chain reaction (PCR) (F3 and B3 LAMP primers were used as the PCR primer pair) were conducted as described by Kuboki, Inoue, Sakurai, Di Cello, Grab, Suzuki, Sugimoto & Igarashi (2003). For PCR, DNA was extracted from filter papers using Qiagen kit according to the manufacture's instructions (QiAamp DNA Mini Kit, Qiagen®; Maryland, USA).

LAMP primer sets designed from HSP70 of *T. parva* amplifies the major bovine *Theileria* spp. except *T. orientalis* DNA (Fig. 1A) and are highly sensitive for the detection of *Theileria* DNA (Fig. 1B). These primers can therefore be used as universal primers for gene amplification of bovine *Theileria* spp. DNA. LAMP primers for *T. congolense* are also highly sensitive and specific (O.M.M. Thekisoe & N. Inoue, unpublished data 2006).

During the collection of the samples in Arusha, no ticks were observed on the sampled animals which probably explains the absence of *Theileria* spp. infections by diagnostic methods used in this study (Table 1). There were no antibodies detected by the indirect fluorescent antibody test (IFAT) against *Theileria* spp. in seven serum samples collected from animals in Arusha (data not shown). In contrast, the fact that *Theileria* spp. were detected from the Dar es Salaam samples (Table 1) is not surprising as bovine *Theileria* infections are common in this region (Msami 2001; Swai, French, Karimuribo, Fitzpatrick, Bryant, Brown & Ogden 2005). It has also been reported that animals which survive *T. parva* infections remain reservoirs of the parasite (Swai *et al*. 2005). Our study revealed a low prevalence of *T. congolense* infections in Arusha (Table 1). However, antibodies against *T. congolese* were detected using enzyme-linked immunosorbent assay ELISA (Bannai, Sakurai, Inoue, Sugimoto & Igarashi 2003) from five out of seven serum samples collected from animals with a previous history of trypanosome infections in Arusha (data not shown). There was a significantly higher prevalence of *T. congolense* infections detected by LAMP and PCR in Dar es Salaam as compared to Arusha (Table 1).

TABLE 1 Detection performance of LAMP, PCR and thin blood smears

Method	Arusha		Dar es Salaam	
	***Theileria* spp.** **+* (%)**	***T. congolense*** **+* (%)**	***Theileria* spp.** **+* (%)**	***T. congolense*** **+* (%)**
LAMP	0/24 (0.0)	4/24 (16.6)	18/40 (45)	12/40 (30.0)
PCR	0/24 (0.0)	1/24 (4.1)	9/40 (22.5)	0/40 (0.0)
TBS**	0/24 (0.0)	0/24 (0.0)	0/40 (0.0)	0/40 (0.0)

+* Number of positively detected samples
** Thin blood smear

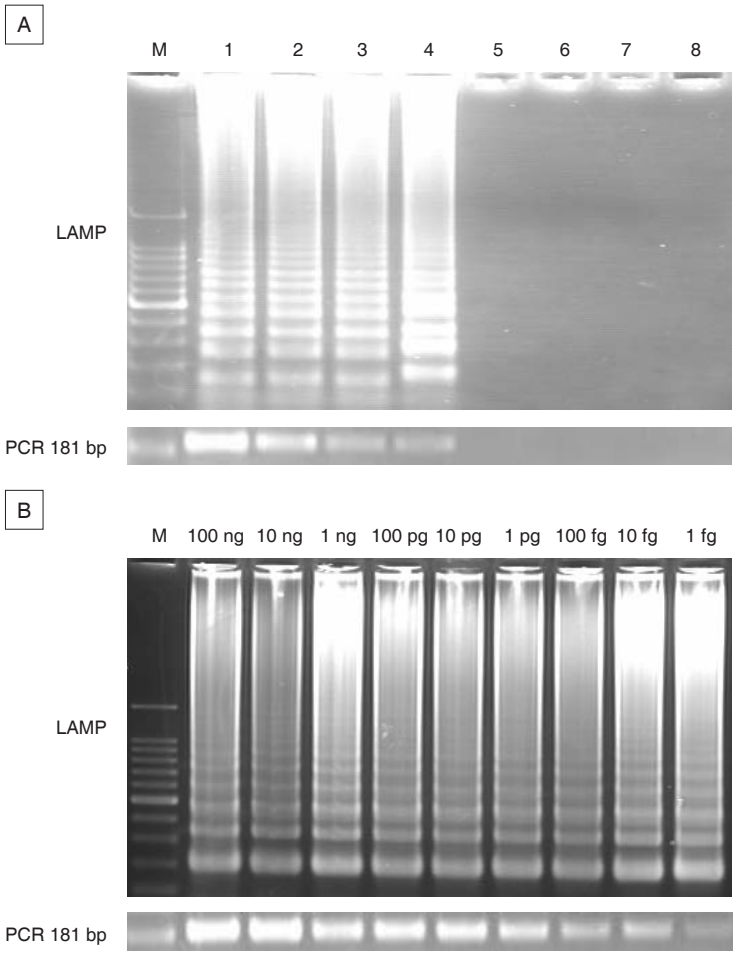

FIG. 1 (A) Specificity tests of LAMP and PCR (F3, B3) primers for detection of bovine *Theileria* spp. DNA. Lanes M: 100 bp maker; 1: *T. parva*; 2: *T. annulata*; 3: *T. taurotragi*; 4: *T. mutans*; 5: *T. orientalis*; 6: *B. bovis*; 7: *T. brucei*; 8: Bovine blood. (B) Sensitivity of LAMP and PCR (F3 and B3) primers for detection of *Theileria parva* DNA quantified with a spectrophotometer (Smart Spec 3000, USA) and serially diluted from 100 ng to 1 fg

This study further confirms the presence of *T. congolense* in sampled areas of Tanzania as previously reported (Malele, Craske, Knight, Ferris, Njiru, Hamilton, Lehane, Lehane & Gibson 2003), which is mainly due to wide distribution of different tsetse fly species infesting about 60 % of the country (Mugittu *et al*. 2001). No parasites were detected by microscopic examination of Giemsa-stained thin blood smears from all the sampled cattle in both Arusha and Dar es Salaam.

In conclusion, LAMP showed higher detection sensitivity than PCR and microscopy, as has previously been reported (Kuboki *et al.* 2003). Furthermore, LAMP can easily amplify DNA templates from filter papers since *Bst* DNA polymerase used in LAMP is not affected by blood-derived tissue components that can inactivate *Taq* DNA polymerase used in PCR (Grab, Lonsdale-Eccles & Inoue 2005). Hence, due to its rapidity, simplicity and high sensitivity, it is suggested that LAMP can be a better molecular diagnostic tool for application in large-scale epidemiological studies of theileriosis and trypanosomosis.

ACKNOWLEDGEMENTS

We are grateful to the farmers in Arusha and Dar es Salaam for their co-operation. We also thank the Director of Veterinary Service, Tanzania, for permission to publish this work. This study was made possible by Program for Promotion of Basic Research Activities for Innovative Biosciences, PROBRAIN, Japan, to NI, and in part by a JSPS grant-in-aid for Scientific Research to NI.

REFERENCES

BANNAI, H., SAKURAI, T., INOUE, N., SUGIMOTO, C. & IGARASHI, I. 2003. Cloning and expression of mitochondrial heat shock protein 70 of *Trypanosoma congolense* and potential use as a diagnostic antigen. *Clinical and Diagnostic Laboratory Immunology*, 10:926–933.

GRAB, D.J., LONSDALE-ECCLES, J. & INOUE, N. 2005. LAMP for tadpoles. *Nature Methods*, 2:635.

KUBOKI, N., INOUE, N., SAKURAI, T., DI CELLO, F., GRAB, D.J., SUZUKI, H., SUGIMOTO, C. & IGARASHI, I. 2003. Loop-mediated isothermal amplification for detection of African trypanosomes. *Journal of Clinical Microbiology*, 41: 5517–5524.

LYNEN, G., LOOMU, P.M., MLINGA, N., KESSY, V.J. & NALITOLELA, S. 2000. Ormilo in indigenous cattle population of the pastoral community. *Proceedings of the 17th Tanzania Veterinary Association Scientific Conference*, *December 2000*, *Arusha International Conference Centre, Arusha, Tanzania.*

MALELE, I., CRASKE, L., KNIGHT, C., FERRIS, V., NJIRU, Z., HAMILTON, P., LEHANE, S., LEHANE, M. & GIBSON, W. 2003. The use of specific and generic primers to identify trypanosome infections of wild tsetse flies in Tanzania by PCR. *Infection, Genetics and Evolution*, 3:271–279.

MORI, Y., NAGAMINE, K., TOMITA, N. & NOTOMI, T. 2001. Detection of loop-mediated isothermal amplification reaction by turbidity derived from magnesium pyrophosphate formation. *Biochemical and Biophysical Research Communications*, 289:150–154.

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*, 94:105–112.

MSAMI, H.M. 2001. An epidemic of East Coast fever on a dairy farm in eastern Tanzania. *Preventive Veterinary Medicine*, 49:55–60.

NOTOMI, T., OKAYAMA, H., MASUBUCHI, H., YONEKAWA, T., WATANABE, K., AMINO, N. & HASE, T. 2000. Loop-mediated isothermal amplification of DNA. *Nucleic Acids Research*, 28:e63.

SAMBROOK, J. & RUSSELL, D.W. 2001. Preparation and analysis of eukaryotic genomic DNA, in *Molecular cloning*, 3rd ed., edited by J. Sambrook & D.W. Russell, Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.

SWAI, E.S., FRENCH, N.P., KARIMURIBO, E.D., FITZPATRICK, J.L., BRYANT, M.J., BROWN, P.E. & OGDEN, N.H. 2005. Spatial and management factors associated with exposure of smallholder dairy cattle in Tanzania to tick-borne pathogens. *International Journal for Parasitology*, 35:1085–1096.