African Journal of Biotechnology Vol. 11(79), pp. 14490-14497, 2 October, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB12.2134
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Polymerase chain reaction (PCR) detection of mixed trypanosome infection and blood meal origin in field-captured tsetse flies from Zambia

Mwandiringana E.[1], Gori E.[1]*, Nyengerai T.[2] and Chidzwondo F.[3]

[1]Department of Preclinical Veterinary Science, University of Zimbabwe, Zimbabwe.
[2]Department of Veterinary Services, Ministry of Agriculture, Zimbabwe.
[3]Department of Biochemistry, University of Zimbabwe, Zimbabwe.

Accepted 27 July, 2012

The prevalence of animal African trypanosomes and source of blood meal in wild field captured tsetse fly was assessed by single polymerase chain reaction using paraflagellar rod protein A (PFRA), protein kinase (KIN) and serum resistance- associated (SRA) primers, respectively for *Trypanozoon* group species, universal trypanosome species and *Trypanosoma brucei rhodesiense.* DNA samples (250) were extracted from tsetse fly collected in South Luangwa National Park in Zambia. Nine host species namely, African buffalo, hippopotamus, giraffe, lion, warthog, African elephant, greater kudu, human and bush pig were revealed in 54% (135) of the samples through amplification and sequencing of cytochrome-b gene. Mixed and individual infection rates in tsetse were successfully determined using a single PCR with KIN primers. Infection rates were highest for *Trypanosoma vivax* 38 (15.2%) followed by T*. brucei* 18 (7.2%), *Trypanosoma congolense* Kenya 16 (6.4%), *T. congolense* savannah 8 (3.2%), and lastly *T. theileri* and *T. congolense* forest, each found in 4 (1.6%) of all tsetse fly. *T. vivax* occurred more frequently in concomitant infections, implying a higher tendency of co-existence. KIN primers were able to amplify multiple trypanosomes DNA from field captured tsetse fly through a single PCR, which makes it a more efficient and cost effective diagnostic method applicable in field situations.

Key words: Tsetse fly, host blood meal, cytochrome-b gene, African animal trypanosomes, nagana, polymerase chain reaction (PCR) technology.

INTRODUCTION

Trypanosomosis is a disease complex caused by several species of protozoan parasites of the genus Trypanosoma. The parasite is transmitted to mammalian hosts by the bite of an infected tsetse fly which causes African sleeping sickness, a fatal disease in humans, and nagana in cattle. From an economic point of view, tsetse-transmitted trypanosomosis is particularly important in cattle where it is mainly caused by *Trypanosoma congolense, T. vivax* and to a lesser extent, *T. brucei brucei* (Morlais et al., 1998). In order to understand the epidemiology of trypanosomiasis, the various species of trypanosomes that infect tsetse fly have to be identified accurately using methods that are highly specific and sensitive. The classical technique for identification of trypanosomes relies on dissection and microscopical examination of the potentially infected tsetse organs such as proboscis, midgut and salivary glands where the parasites will be located (Lloyd and Johnson, 1924). The method is labour intensive and the detection requires large amount of trypanosomes. Furthermore, visual examination does not allow the identification of parasites below the subgenus level and cannot detect immature and mixed infections. Molecular biology methods have to a larger extent overcome the limitations of the old methods.

Introduction of DNA based methods such as species specific DNA probes and group/species specific polymerase chain reaction (PCR) into diagnostic tests for the detection of infection with African trypanosomes in

*Corresponding author. E-mail: egori@science.uz.ac.zw. Tel: +263-4-303211 ext 19048, +263 772 668 578.

humans, animals, as well as in tsetse fly has improved the specificity and sensitivity of parasite detection and identification (Kukla et al., 1987; Gibson et al., 1988; Moser et al., 1989; Masiga et al., 1992; Katakura et al., 1997; Malele et al., 2003; Adams et al., 2008; Konnai et al., 2008;). PCR techniques have been successfully applied in the detection of host blood meal of tsetse fly and identification of their origin through amplification of cytochrome-b gene (Steuber et al., 2005; Mekata et al., 2008; Konnai et al., 2008). The techniques continue to be improved ever since their introduction. Kuboki et al. (2003) reported about enhanced PCR using Ampdirect-D reagent (Shimadzu Biotech Company, Japan) which is capable of effectively neutralizing substances that inhibit blood and tissue derived DNA amplification to amplify the paraflagellar rod protein A (PFRA) gene in order to detect trypanosomes in infected mice blood blotted on filter paper. Desquesnes et al. (2001) also showed that serine/threonine protein kinase (KIN 1 and KIN 2) gene primer set were capable of multiple species diagnosis of the main livestock trypanosomes through a single PCR. In particular, the high prevalence of mixed infections with two, three or even four different trypanosome species was recognized in different studies (Majiwa and Otieno, 1990; McNamara et al., 1989, 1995; Solano et al., 1995; Woolhouse et al., 1996). With the use of species-specific PCR tests, it is now possible to identify the 11 tsetse-transmitted trypanosome species and subgroups for which there are available primers (Adams et al., 2008). Furthermore, PCR using serum resistance-associated (SRA) gene primers which amplify specifically *T. b. rhodesiense* (Radwanska et al., 2002) has been used to distinguish *Trypanozoon* group species *T. b. rhodesiense* from *T. b. brucei* and *T. b. gambiense* infections.

Desquesnes et al. (2001) successfully used KIN primers to amplify trypanosomes in African livestock using cattle buffy coat samples in a single PCR. However, the sensitivity for *T. vivax* was very low, raising the possibility that the sample source could have affected the detection rate. The current study postulated that KIN primers can be used to detect multiple trypanosome infections in field captured tsetse fly in a single reaction. This study applied the afore mentioned method to detect natural trypanosome infections as well as mammalian host DNA in wild, field captured tsetse fly. The results of this study contribute towards the understanding of the epidemiology of trypanosomosis hence adjustment and modification of the existing control methods which are often complicated by mixed infections.

MATERIALS AND METHODS

Study site and tsetse fly collection

Tsetse flies were captured in South Luangwa National Park in September 2008. South Luangwa National Park was chosen because of relative abundance of a diverse wildlife population and a high population density of *Glossina* species. The area has a good ecological niche for trypanosomiasis transmission between tsetse and mammals. The area has mean annual rainfall of 800 mm and an altitude of 500 to 600 m. Daily ambient temperatures range from 32 to 36°C, with mean minimum daily temperatures of 16 to 23°C, respectively. Tsetse flies were caught using baited NG2B traps as described by Brightwell et al. (1991). Six traps were deployed at 200 m apart and tsetse fly captured at 24 h intervals over three days. All traps were baited with acetone released from a 6 mm diameter hole in the top lid of a half liter plastic container. 250 samples of Glossina species were used in the study. After collection from the field, tsetse samples were wrapped in polythene paper and stored at 4°C prior to DNA extraction.

DNA extraction and pooled sample preparation

DNA was separately extracted from individual tsetse fly giving a total of 250 isolated DNA samples. Tsetse flies were disrupted by grinding in a Biomasher® tissue homogenizer unit (Funakoshi Company, Japan) in accordance with the manufacturer's protocol. A 100 µl detergent lysis buffer [10 mM Tris-HCl (pH 8.0), 100 mM EDTA (pH 8.0), 10% sodium dodecyl sulphate] was added, together with 100 µg/ml of proteinase K (25 µl) followed by overnight incubation at 55°C. The established phenol-chloroform-isoamyl alcohol DNA extraction was done as described by Sambrook and Russell (2001). DNA was precipitated in isopropanol; air dried and then dissolved in 200 µl of sterilized distilled water. The DNA samples were kept frozen at -20°C, only to be thawed just before performing PCR amplifications.

Detection of trypanosomes using PFRA, KIN and SRA primers

The 250 DNA samples were randomly placed into groups of ten each, making a total of 25 groups of pooled DNA samples. Prior to pooling, each individual DNA sample was diluted. Each sample contributed 5 µl of DNA template which was mixed with others with the same concentration to make a total volume of 50 µl pooled DNA sample. Pooling of DNA was done in order to screen the whole study population for the presence of trypanosomes and mammalian cytochrome-b gene in minimal number of reactions and therefore exclude negative samples in subsequent analyses, since not all tsetse were infected. The resulting 25 pooled samples were subjected to PCR amplification for detection of trypanosome infections and mammalian cytochrome-b. Only individual samples that were part of DNA pools that produced a positive PCR result were screened for trypanosome infection and mammalian cytochrome-b.

Samples were subjected to three different amplification reaction conditions (Table 1) to detect *Trypanozoon* group species, universal trypanosome and *T. b. rhodesiense* parasites using PFRA, KIN and SRA primers, respectively. PCR was performed in 25 µl volumes consisting of 5 µl pooled DNA, 20 pmol of specific primers (sequence shown in Table 2) in the presence of 0.25 µl of *Taq* DNA polymerase (Invitrogen, USA) and 12.5 µl of 2× Ampdirect® Plus buffer (Shimadzu Biotech Company, Tsukuba, Japan), 1.5 mM $MgCl_2$ and 0.2 mM of dNTPs. All samples positive for both the *Trypanozoon* group and *T. brucei* using the PFRA and KIN primers, respectively were subjected to PCR for the detection of *T. b. rhodesiense.* Reagent preparations were the same as for PFRA and KIN except that in this reaction, NovaTaq™ hot start DNA polymerase (Novagen, Madison, WI) was used. The PCR products were monitored by electrophoresis on 2% agarose gel stained with ethidium bromide. In all reactions, distilled water was the non DNA template control while *T. evansi* DNA, *T. b. gambiense* isolate DNA, *T. b. rhodesiense* isolate IL1501 DNA and *Syncerus caffer* DNA were used as positive controls for PFRA, KIN,

Table 1. PCR parameters for detection of trypanosome infections and origin of blood meal.

PCR profile	PFRA	KIN	SRA	Cytochrome-b
Pre-denaturation	95°C (10 min)	94°C (3 min)	94°C (10 min)	95°C (5 min)
Denaturation	95°C (45 s)	94°C (45 s)	94°C (60 s)	94°C (30 s)
Annealing	57°C (60 s)	58°C (30 s)	68°C (60 s)	52°C (30 s)
Extension	72°C (60 s)	72°C (60 s)	72°C (60 s)	72°C (45 s)
Number of cycles	35	35	35	35
Further extension	72°C (7 min)	72°C (10 min)	72°C (10 min)	72°C (5 min)

Table 2. Trypanosome species specific primers used in this study.

Gene	Specific primer	Sequence	Reference
Trypanozoon group	PFRA F3 PFRA B3	5'-TCACAACAAGACTCGCACG-3' 5'-GGGCTTTGATCTGCTCCTC-3'	Kuboki et al. (2003)
Universal trypanosome species	KIN 1 KIN 2	5'-GCGTTCAAAGATTGGGCAAT-3' 5'-CGCCCGAAAGTTCACC-3'	McLaughlin et al. (1996)
T. b. rhodesiense	SRA F SRA R	5'-TAGTGACAAGATGCGTACTCAACGC-3' 5'-ATGTGTTCGAGTACTTCGGTCACGCT-3'	Radwanska et al. (2002)
Mammalian cytochrome-b	CYTB 1 CYTB 2	5'-CCATCCAACATCTCAGCATGATGAAA-3' 5'-GCCCCTCAGAATGATATTTGTCCTCA- 3'	Steuber et al. (2005)

Table 3. Frequency of trypanozoon group and trypanosome infections in tsetse fly detected using PFRA and KIN primers.

PFRA primer	KIN primer					
Trypanozoon group	*T. brucei*	*T. c.* forest	*T. c.* savannah	*T. c.* Kenya	*T. vivax*	*T. theileri*
53/250 (21.20%)	8/250 (7.20%)	4/250 (1.60%)	8/250 (3.20%)	16/250 (6.40%)	38/250 (15.2%)	4/250 (1.60%)

SRA and cytochrome-b primer reactions, respectively. Product size was determined by graphic intrapolation on the photographs based on the molecular supper ladder marker 100®.

Detection of blood meal origin by amplification of mammalian cytochrome-b

250 samples were placed into groups of ten each, with each sample in a particular group contributing 5 µl of DNA solution to the 50 µl pooled DNA sample. Pooled DNA was used to screen mitochondrial cytochrome-b gene by PCR (see table for reaction conditions). Individual DNA sample contributing to the positive pool were subjected to independent PCR reactions similar to those for pooled samples except that 3 µl of DNA solution was used as the template DNA. The observed cytochrome-b positive PCR product was purified using the QIAquick PCR Purification Kit and protocol (Qiagen, Germany) according to manufacturer's instructions before sequencing. The purified products were then directly sequenced using the Big Dye Terminator Cycle. The cycle sequence reactions were prepared using Big-Dye Terminator mix (Applied Biosystems, Foster City, CA) according to the manufacturer's protocol. Sequence analysis using the National Center for Biotechnology Information Basic Local Alignment Search Tool (NCBI BLAST) (http://blast.ncbi.nlm.nih.gov) analysis to find the most similar sequences was done to identify blood meal origin vertebrate species. Further confirmation of BLAST was done through manual individual alignment of obtained sequences with existing vertebrate ones, using Clustal W DNA sequence alignment software (http://npsa-pbil.ibcp.fr).

RESULTS

Detection of trypanosome species in tsetse fly derived DNA

PCR detection of trypanosomes using universal KIN primers revealed three *T. congolense* subgroups that is, *T. congolense* Kenya, *T. congolense* forest and *T. congolense* savannah, as well as other trypanosome species namely *T. brucei*, *T. vivax* and Infection rates ranged from 1.6 to 15.2%, with *T. vivax* and *T. brucei* being the most infecting trypanosomes (Table 3). Mixed infections with varying trypanosome species

Table 4. Frequency of mixed trypanosome infections

Species	Frequency
T. congolense Kenya/*T. congolense* savannah	1
T. congolense Kenya/*T. brucei*	2
T. congolense Kenya/*T. vivax*	5
T. congolense forest/*T. brucei*	2
T. congolense savannah/*T. vivax*	1
T. congolense Kenya/*T. congolense* forest	1
T. brucei/T. vivax	4
T. brucei/T. theileri	1
T. vivax/T. theileri	1
T. congolense forest/*T. brucei/T. vivax*	1
T. brucei/T. theileri/T. vivax	1
Total	20

Table 5. Host blood meal origin determined by similarity with sequences of species in the GenBank database.

Host species	Mitochondrial DNA detection rate	Similarity (%)
Syncerus caffer (African buffalo)	58/135 (43.0%)	98
Giraffa camelopardalis (Giraffe)	10/135 (7.4%)	97
Homo sapiens (Human)	6/135 (4.4%)	99
Hippopotamus amphibius (Hippo)	23/135 (17.0%)	97
Loxodonta africana (African elephant)	23/135 (17.0%)	97
Tragelaphus strepsiceros (Greater Kudu)	8/135 (5.9%)	97
Phacochoerus africanus (Warthog)	3/135 (2.2%)	95
Panthera leo (Lion)	1/135 (0.7%)	98
Mixed host cases		
Buffalo/bush pig, buffalo/elephant and Buffalo/lion	3/135 (2.2%)	≥95

A match of ≥ 95% was considered.

combinations were detected and are shown in Table 4. Frequencies of trypanosomes and host species were recorded (Tables 3 and 4) based on results of individual tsetse fly DNA samples that were analysed separately.

Detection of *T. b. rhodesiense* using SRA primers

Out of the 53 samples positive for the *Trypanozoon* group through PCR with PFRA gene primers, none were positive for *T. b. rhodesiense*. Also, of the 18 samples positive for *T. brucei* with KIN primers, none were positive for *T. b. rhodesiense.*

Detection of blood meal origin

135 samples (54%) were positive for the mammalian mitochondrial cytochrome-b gene. Sequence analysis and alignment of the 135 sequences established nine different host species namely *Syncerus caffer* (African buffalo), *Giraffa camelopardalis* (Giraffe), *Potamochoerus porcus* (Bush pig), *Hippopotamus amphibius* (Hippo), *Phacochoerus africanus* (Warthog), *Panthera leo* (Lion), *Tragelaphus strepsiceros* (Greater kudu), *Loxodonta africana* (African elephant) and *Homo sapiens* (Human). Detection rates (Table 5) ranged from 0.7 to 43.0%.

Three samples had two host species which were; buffalo/bush pig, buffalo/elephant and buffalo/lion combinations accounting for 2.2% of cytochrome-b positive samples. Possible reservoir hosts for the detected trypanosome species was performed by combination of information obtained from both vertebrate host detection and trypanosome detection, and the observed results are shown in Table 6. Types of trypanosome species found in vertebrates ranged from none in *P. leo* to all detected in *S. caffer. S. caffer* and *H. amphibious* were the most predominant host reservoirs

Table 6. Host species of trypanosome infected tsetse fly.

Host species	*Trypanozoon* group (%) N=53	*T. b. brucei* (%) N=18	*T. c. forest* (%) N=4	*T. c. savannah* (%) N=8	*T. c. Kenya* (%) N=16	*T. vivax* (%) N=38	*T. theileri* (%) N=4
S. caffer African buffalo	10/53(18.9)	3/18(16.7)	1/4(25)	1/8(12.5)	6/16(37.5)	8/38(21.1)	2/4(50)
G. camelopardalis (Giraffe)	0	1/18 (5.6)	0	0	0	0	0
Homo sapiens (human)	1/53(1.9)	0	0	0	1/16(6.25)	1/38(2.6)	0
H. amphibius (Hippo)	6/53(11.3)	1/18(5.6)	1/4(25)	2/8(25)	4/16(25)	3/38(7.9)	0
L. africana (African elephant)	6/53(11.3)	2/18(11.1)	0	1/8(12.5)	1/16(6.25)	6/38(15.8)	0
T. strepsiceros (Greater Kudu)	1/53(1.9)	2/18(11.1)	1/4(25)	0	0	2/38(5.3)	0
P. africanus (Warthog)	1/53(1.9)	0	0	0	1/16(6.25)	1/38(2.6)	0
P. Leo (Lion)	0	0	0	0	0	0	0
S. caffer/*P. porcus* (Buffalo/Bush pig)	1/53(1.9)	1/18(5.6)	0	0	0	0	0

for the trypanosomes.

DISCUSSION

Natural trypanosome infections have been detected in several wild animals at varying rates in free living wild animals (Baker, 1968; Mbaya et al., 2008, 2009). Trypanosome infections were detected in 6.3% of 1242 game animals of varying species in some African countries (Ashcroft, 1959; Geigy et al., 1967; Tarimo et al., 1991). In several studies, wild ungulates have been reported to serve as reservoir hosts for pathogenic animal African trypanosomes (Baker et al., 1967; MMH, 1987; Connor, 1994; Mbaya et al., 2009). On the other hand, primates and antelopes were identified as potential reservoirs for human infective trypanosomes such as *T. brucei gambiense* and *T. brucei rhodesiense* (Kaguraka, 1992). Although wild animals have been shown to play a reservoir host role for the human infective *T. b. rhodesiense* in East and Southern Africa (Welburn et al., 2001a, b; Van den Bossche et al., 2005), none of the tested samples were positive, hence all the *T. brucei* infections can be attributed to *T. b. brucei*. The finding is in agreement with Kaguraka (1992) who noted that of the wild animals, it is the primates and antelopes in particular, that serve as reservoir hosts for human infective trypanosomes. Thus, the negative result for *T. brucei rhodesiense* found in this study is in agreement with this observation. No wild primate or antelope were detected as hosts in the current study. The reported lack of *T. b. rhodesiense* could also be due to sensitivity of the PCR test on SRA gene, since the gene occurs as a single copy unlike PFRA A gene which has about 28 copies. Desquesnes and Davila (2002) noted that sensitivity is reduced for tests targeting genes with low copy numbers as compared to those with multiple copies such as satellite DNA with over 10000 copies.

In the current study, the prevalence of *T. b. brucei* using the PFRA primers was 21.2%, which is 2.9% higher compared to that reported by Konnai et al. (2008) which was at 18.3%. Infection rates for four main livestock infective trypanosomes were obtained by PCR using the KIN primers, further demonstrating the ability of KIN 1 and KIN 2 primer set to detect and differentiate all livestock trypanosome through a single PCR (Desquesnes et al., 2002). *T. vivax* had the highest prevalence followed by *T. b. brucei, T. congolense* Kenya*, T. congolense* savannah, then both *T. congolense* forest and *T. theileri* were the least in that order. Mixed infections with two or three different trypanosome species were common, this phenomenon is consistent with findings reported by Konnai et al. (2008), Lehane et al. (2000), Van den Bossche et al. (2004) and Masiga et al. (1996) but most of these studies used more than a single PCR with different sets of primers specific for particular species. The present used single PCR, however, on agarose gel, it was challenging to distinguish between bands of mixed infections of *T. congolense* forest (714 bp) with *T. congolense* and *T. congolense* savannah (697 bp) with *T. congolense* Kenya, due to their close proximity and small difference of 30 base pairs in size (Figure 1).

The common single infections were the *T. congolense* savannah 6/8 and *T. vivax* (26/38) while the least common was *T. congolense* forest which only occurred together with other trypanosomes (twice with *T. brucei,* once with *T. congolense* Kenya and once in a triple infection

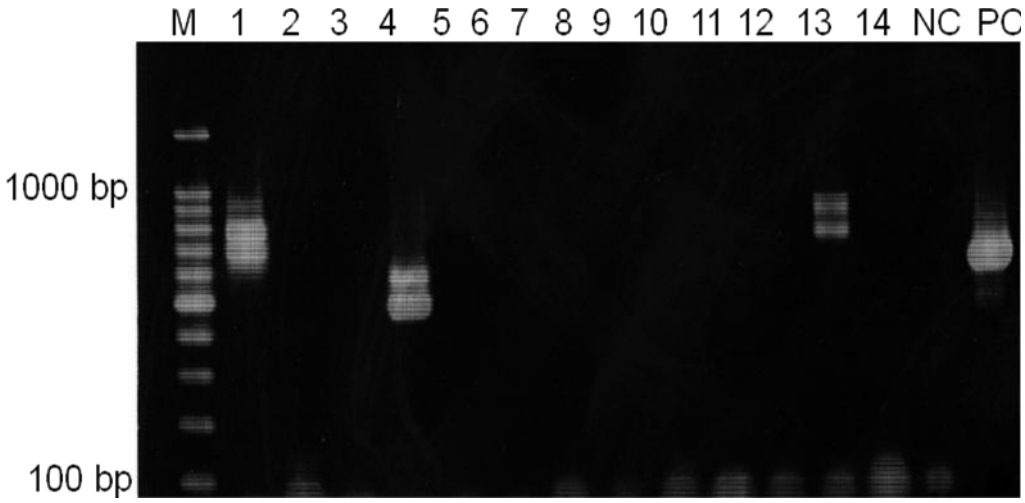

Figure 1. Multiple trypanosome species detection by a single PCR using KIN primer set. Lane M is the 100 bp marker; Lane 1, 4 and 13 is the PCR product showing mixed infections with; 1-*T. congolense* Kenya and *T. congolense* savannah, 4-*T. brucei* and *T. theileri* and 13-*T. congolense* Kenya and *T. congolense* forest*;* Lanes 2, 3, 5, 6, 7, 8, 9, 10, 11, 12 and 14 are negative samples; NC is the negative control and PC is the *T. b. gambiense* positive control.

with *T. brucei* and *T. vivax*). The common pattern of double infections was for *T. vivax* and T. congolense Kenya (5/20) and *T. brucei/T. vivax* (4/20). Other double infections which occurred once were for *T. congolense* Kenya*/T.congolense* savannah, *T. congolense* savannah*/ T. vivax, T. brucei/T. theileri, T. vivax/T. theileri,* and twice for *T. congolense* Kenya*/T. brucei. T*. *congolense* Kenya/*T*. *vivax* combination had the highest frequency followed by *T*. *brucei*/*T*. *vivax* combination. Mixed infection trypanosome species with the highest frequency were found between different species whose development and/or transmission takes place in different parts of the tsetse fly. The explanation to this observation could be that since the trypanosome species do not occupy the same parts in the tsetse fly for development and/or transmission, competition for survival between them is less than that of species which develop and/or are transmitted from the same parts of the fly. The drawback of the PCR method is that it does not enable us to determine whether all the species present in one such fly reach an infective stage (Mekata et al., 2008). It would be also important to determine co-infections using sera from animals in order to determine whether infection rates obtained in tsetse fly correspond to the infection rates in animals. Furthermore, it would be recommended to determine host, trypanosome and tsetse fly species preference using the same technique in order to get a full understanding of their interactions in a given niche. The use of universal tests for pathogenic trypanosomes demonstrated in this study is of great diagnostic and economic importance as it reduces the cost of PCR by three to five times owing to reduced number of reactions required per sample, one without compromising test results (Desquesnes and Davila, 2002).

Polymerase chain reaction was successfully used to amplify the conserved regions of the mitochondrial gene cytochrome-b (359 bp) from vertebrate hosts of wild captured tsetse flies using one set of primers. Kocher et al. (1989) reported that universal primers could be used as complements to the conserved region of the mitochondrial cytochrome-b gene in vertebrates as has been achieved in this study. The attribute of high copy number makes PCR amplification of mitochondrial cytochrome-b DNA genes a robust option for analyzing vertebrate DNA in tsetse and other arthropod blood meals (Solano et al., 1995). In this study, consensus sequences of the cytochrome-b gene were used as the basis for detection of biological origin of blood meal, and matches were obtained with more than 95% similarities (Table 5). Samples with human DNA showed the highest match with 99% sequence similarity. Previous work done by Konnai et al. (2008) and Mekata et al. (2008) showed that sequence analysis of the vertebrate mitochondrial amplicons established that originated from eight (human, African elephant, African buffalo, water buck, roan antelope, greater kudu, warthog and goat) and seven (human, elephant, buffalo, goat, warthog, greater kudu and cattle) different vertebrate species, respectively and also support the evidence obtained in this study where African buffalo, bush pig, giraffe, lion, warthog, hippopotamus, African elephant, greater kudu and human DNA were detected in the blood meal of tsetse flies.

Accumulating evidence show that in a particular area infested with tsetse, the fly tend to have a dominant vertebrate from which they obtain blood meal. In this study, the dominant host with the highest mammalian cytochrome-b detection rate was the buffalo. This is similar to reports of a dominant host in studies by Konnai

et al. (2008) and Mekata et al. (2008), where the human was the dominant host. These findings suggest that the tsetse fly in this area have a preference for buffalo which would therefore seem to support earlier reports on the subject (Clausen et al., 1998; Simo et al., 2008). However, this preference only applies to the particular area from where tsetse flies were collected and cannot be used to make a general host preference conclusion on tsetse flies in Zambia. Host preference varies from place to place when incorporating other factors such as tsetse fly species involved, geographical location and animal population. Furthermore, tsetse flies were also found to harbor blood meals from more than one host, a phenomenon common to earlier studies (Konnai et al., 2008; Mekata et al., 2008) and the present work, suggesting that it may be possible for the fly to act as a bridging vector for trypanosome transmission from one animal species to the next. Torr et al. (2001) reported inability to detect the cytochrome-b gene in unfed flies, therefore, a negative result in this current study could mean that the flies had not had a blood meal or could have been captured more than 96 h post feeding, hence the blood meal may have been completely degraded or in concentrations too low to be detected by PCR (Steuber et al., 2005).

In an attempt to investigate on the main reservoir host of each trypanosome species detected in this study, data on vertebrate host and trypanosome species detected from each fly were integrated. Across all trypanosome species except *T. congolense* savannah where the Hippo is dominant, the dominant host was the buffalo, which might lead to a conclusion that it could be the main trypanosome reservoir host in South Luangwa National Park in Zambia. In conclusion, the findings of the present study show that the KIN primers have the ability to detect multiple species in a single PCR from field tsetse fly samples collected in South Luangwa National Park in Zambia.

ACKNOWLEDGEMENTS

This work was supported by the Japan International Cooperation Agency (JICA) in collaboration with the National Research Center for Protozoan Diseases (NRCPD), Obihiro University of Agriculture and Veterinary Medicine.

REFERENCES

Adams E, Hamilton PB, Malele I, Gibson WC (2008). The identification, diversity and prevalence of trypanosomes in field caught tsetse in Tanzania using ITS-1 primers and fluorescent fragment length barcoding. Infect. Genet. Evol. 8:439-444.

Ashcroft MT (1959). The importance of African wildlife as reservoir of trypanosomosis, East Afr. Med. J. 36: 289–297.

Baker JR (1968). A case of *Trypanosoma congolense* in an African lion and leopard. In: Transactions of the Royal Society of Tropical Medicine and Hygiene (1967), London, pp 62–85.

Baker JR, Sachs R, Laufer I (1967). Trypanosomes of wild mammals in an area northwest of the Serengeti National Park, Tanzania. Zoologic. Trop. Med. Parasitol. 18: 280–284.(was not cited in the article).

Brightwell R, Dransfield RD, Kyorku CTK, Tarimo AA, Mundai D (1991). Development of a low-cost tsetse trap and odour baits for *Glossina pallidipes* and *G. longipennis* in Kenya. Med. Vet. Entomol. 5(2):153-64.

Clausen PH, Adeyemi I, Bauber B, Breloeer M, Salchow F, Staak C (1998). Host preferences of tsetse (Diptera: Glossinidae) based on blood meal identifications. Med. Vet. Entomol. 12:169-180.

Connor RJ (1994). The impact of nagana, Onderstepoort. J. Vet. Res. 61:379–383.

Desquesnes M, Davila AM (2002) Applications of PCR-based tools for detection and identification of animal trypanosomes: a review and perspectives. Vet. Parasitol. 11:213–231

Desquesnes M, McLaughlin G, Zoungrana A, Dávila AMR (2001). Detection and identification of *Trypanosoma* of African livestock through a single PCR based on internal transcribed spacer 1 of rDNA. Int. J. Parasitol. 31:610-614.

Geigy R, Kauffman M, Beglinger (1967). A survey of wild animals as potential reservoirs of trypanosomosis in the Ulanga district (Tanzania), Acta Tropica 24:97–108.

Gibson WC, Dukes P, Gashumba JK (1988). Species-specific DNA probes for the identification of trypanosomes in tsetse. Parasitology. 97:63-73.

Kaguraka P (1992). Concepts on the existence of an animal reservoir for *Trypanosoma brucei gambiense*. Parasitol. Res. 16:30–44.

Katakura K, Lubinga C, Chitambo H, Tada Y (1997). Detection of *Trypanosoma congolense* and *T. brucei* subspecies in cattle in Zambia by polymerase chain reaction from blood collected on a filter paper. Parasitol. Res. 83:241-245.

Kocher TD, Thomas WK, Meyer A, Edwards SV, Pääbo S, Villablanca F X, Wilson A C (1989). Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. Nat. Acad. Sci. USA. 86:6196-6200.

Konnai S, Mekata H, Odbileg R, Simuunza M, Chembensof M, Witola W H, Tembo M E, Chitambo H, Inoue N, Onuma M, Ohashi K (2008). Detection of *Trypanosoma brucei* in field-captured tsetse flies and identification of host species fed on by the infected flies. Vector Borne Zoonotic Dis. 8:1-9.

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. J. Clin. Microbiol. 41:5517-5524.

Kukla BA, Majiwa PAO, Young CJ, Moloo SK, Ole-Moiyoi OK (1987). Use of species specific DNA probes for the detection and identification of trypanosome infections in tsetse flies. Parasitology. 95:1-26.

Lehane MJ, Msangi AR, Whitaker CJ, Lehane SM (2000). Grouping of trypanosome species in mixed infections in *Glossina pallidipes*. Parasitology. 120:583-592.

Lloyd L, Johnson WB (1924). The trypanosome infections of tsetse flies in Northern Nigeria and a new method of estimation. Bull. Entomol. Res. 14:265–288.

Majiwa PAO, Otieno LH (1990). Recombinant DNA probes reveal simultaneous infection of tsetse flies with different trypanosome species. Mol. Biochem. Parasitol. 40:245–254.

Ministry of Health (MMH) (1987). Trypanosomiasis, Malawi. Epidemiologic. Quart.1.

Malele I, Craske L, Knight C, Ferris V, Njiru Z, Hamilton P, Lehane S, Lehane M, Gibson WC (2003). The use of specific and generic primers to identify trypanosome infections of wild tsetse flies in Tanzania by PCR. Infect. Genet. Evol. 3:271-279.

Masiga DK, McNamara JJ, Laveissière C, Truc P, Gibson WC (1996). A high prevalence of mixed trypanosome infections in tsetse flies in Sinfra, Côte d'Ivoire, detected by DNA amplification. Parasitology 112:75-80.

Masiga DK, Smyth AJ, Hayes PJ, Bromidge TJ, Gibson WC (1992). Sensitive detection of trypanosomes in tsetse flies by DNA amplification. Int. J. Parasitol. 22:909–918.

Mbaya AW, Aliyu MM, Ibrahim UI (2009). The clinico-pathology and mechanisms of trypanosomosis in captive and free-living wild

animals: A review. Vet. Res. Commun. 33:793–809.
Mbaya AW, Aliyu MM, Nwosu, CO, Ibrahim UI (2008). Captive wild animals as potential reservoirs of haemo and ectoparasitic infections of man and domestic animals in the arid-region of Northeastern Nigeria. Vet. Achiv. 78 (5):429–440.
McLaughlin GL, Ssenyonga SS, Nanteza E, Rubaire-Akiki, Wafula O, Hansen RD, Vodkin MH, Novak RJ, Gordon VR, Montenegro-James S, James M, Aviles H, Armijos R, Santrich C, Weigle K, Saravia N, Wozniak E, Gaye O, Mdachi R, Shapiro SZ, Chang KP, Kakoma I (1996). PCR- based detection and typing of parasites. pp. 261-287. *In*: Parasitology for the 21st Century. Aczel MA, Alkan MZ (Eds.), CAB International, Wallingford Oxon.
McNamara J, Dukes P, Snow WF, Gibson WC (1989). Use of DNA probes to identify *Trypanosoma congolense* and *T. simiae* in tsetse from The Gambia. Acta Trop. 46:55–61.
McNamara JJ, Laveissiere C, Masiga DK (1995). Multiple trypanosome infections in wild tsetse in Cote d'Ivoire detected by PCR analysis. Acta Trop. 59:85–92.
Mekata H, Konnai S, Simuunza M, Chembensofu M, Kano R, Witola WH, Tembo ME, Chitambo H, Inoue N, Onuma M, Ohashi K (2008). Prevalence and source of trypanosome infections in field captured vector flies (*Glossina pallidipes*) in Southeastern Zambia. J. Vet. Med Sci. 70:923-928.
Morlais P, Grebaut JM, Bodo S, Djoha G, Cuny S, Herde (1998). Detection and identification of trypanosomes by polymerase chain reaction in wild tsetse flies in Cameroon. Acta Trop. 70:109–117.
Moser DR, Cook GA, Ochs DE, Bailey CP, McKane MR, Donelson JE (1989). Detection of *Trypanosoma congolense* and *Trypanosoma brucei* subspecies by DNA amplification using the polymerase chain reaction. Parasitology 99:57-66.
Radwanska M, Chamekh M, Vanhamme L, Claes F, Magez S, Magnus E, De Baetselier P, Buscher P, Pays E (2002). The serum resistance-associated gene as a diagnostic tool for the detection of *Trypanosoma brucei rhodesiense*. Am. J. Trop Med. Hyg. 67:684-690.
Sambrook J, Russell DW (2001). Preparation and analysis of eukaryotic genomic DNA. pp 6.1-6.30. *In* Molecular cloning, 3rd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
Simo G, Njiokou F, Mbida Mbida JA, Njitchouang GR, Herder S, Asonganyi T, Cuny G (2008). Tsetse fly host preference from sleeping sickness foci in Cameroon: epidemiological implications. Infect. Genet. Evol. 8:34-39.
Solano P, Argiro L, Reifenberg JM, Yao Y, Duvallet G (1995). Field application of the polymerase chain reaction (PCR) to the detection and characterization of trypanosomes in *Glossina longipalpis (*Diptera*: Glossinidae)* in Cote d'Ivoire. Mol. Ecol. 4:781–785.
Steuber S, Abdel-Rady A, Clausen PH (2005). PCR- RFLP analysis: a promising technique for host species identification of blood meals from tsetse flies (Diptera: *Glossinidae*). Parasitol Res. 97:247-254.
Tarimo NS, Njau BC, Otieni LH (1991. Epizootiology of trypanosomosis in Lambabwe valley, Kenya, East Africa. Insect. Applic. 12:319–384.
Torr SJ, Wilson PJ, Schofield TNC, Mangwiro S, Akber S, White BN (2001). Application of DNA markers to identify the individual-specific hosts of tsetse feeding on cattle. Med. Vet. Entomol. 15:78-86.
Van den Bossche P, De Deken R, Brandt J, Geerts S, Geysen D, Berkvens D (2004). The transmission of mixed *Trypanosome brucei brucei*/*T. congolense* infections by tsetse (*Glossina morsitans morsitans*). Vet. Parasitol. 119:147-153.
Van den Bossche P, Ky-Zerbo A, Brandt J, Marcotty T, Geerts S, De Deken R (2005). Transmissibility of *Trypanosoma brucei* during its development in cattle. Trop Med. Int. Health. 10:833-839.
Welburn SC, Fèvre EM, Coleman PG, Odiit M, Maudlin I (2001a). Sleeping sickness: a tale of two diseases. Trends. Parasitol. 17:19-24.
Welburn SC, Picozzi K, Fèvre EM, Coleman PG, Odiit M, Carrington M, Maudlin I (2001b). Identification of human-infective trypanosomes in animal reservoir of sleeping sickness in Uganda by means of serum-resistance-associated (SRA) gene. Lancet. 358:2017-2019.
Woolhouse MEJ, McNamara JJ, Hargrove JW, Bealby KA (1996). Distribution and abundance of trypanosome (subgenus *Nannomonas*) infections of the tsetse fly *Glossina pallidipes* in Southern Africa. Mol. Ecol. 5:11–18.