FULL PAPER *Parasitology*

A PCR-based survey of animal African trypanosomosis and selected piroplasm parasites of cattle and goats in Zambia

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ABSTRACT. We screened cattle and goats from the districts of Chama, Monze and Mumbwa in Zambia for animal African trypanosomes, *Babesia bigemina* and *Theileria parva* using PCRs; 38.1% of the samples tested positive for at least one of the parasite species. The most common parasite was *Trypanosoma vivax* (19.8%). Its incidence was significantly higher in goats than in cattle, ($P<0.05$). *B. bigemina* was found in samples from all the three areas, making it the most widespread of the parasites in Zambia. Among the tested samples, 12.0% of the positive samples were mixed infections. There were significant differences in the infection rates of *T. vivax* (Mumbwa had a significantly higher infection rate [39.6%, $P<0.0001$]), *Th. parva* (Monze had the only cases [$P<0.0004$]) and *B. bigemina* (Monze had a significantly higher infection rate [40.5%, $P<0.0001$]). According to the hematocrit values, the packed cell volume (%) among the cattle with mixed infections was significantly lower than that of the other cattle. The presence of multiple parasite species and mixed infections among the Zambian cattle and goat populations is of both clinical and economic importance to livestock farming. The absence of trypanosomosis among the samples from Monze can be attributed to tsetse eradication efforts that took place around Lake Kariba. This shows that the prevention and control of these parasitic diseases can have a significant impact on the disease status, which can translate directly into the improvement of the livestock sector in Zambia.

KEY WORDS: animal African trypanosomosis, cattle, goat, piroplasmosis, Zambia

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Hemoprotozoan parasites, which include different species of *Theileria, Babesia* and *Trypanosoma*, often present a challenge to successful livestock farming [25]. *Theileria* and *Babesia* species are among the major piroplasms that affect cattle and small ruminants [7]. Tick-borne diseases cause significant economic losses in the tropical and sub-tropical regions of the world [1, 11]. Among the *Babesia* species, *Babesia bovis* and *B. bigemina* are clinically and economically important; both species are widely distributed in tropical and sub-tropical countries [4]. *Theileria annulata* and *Th. parva*, the two most lymphoproliferative *Theileria* parasites, are considered to be the most pathogenic to cattle [18]. *Th. parva* is prevalent in Eastern, Central and Southern Africa [31]. Animal African trypanosomosis (AAT) affects all domestic animals and a wide range of wildlife species, which act as an infection reservoir for both humans and domestic animals [2]. In cattle, the disease is caused by *T. vivax*, *T. congolense* and *T. brucei brucei* [14]. These trypanosomes are transmitted cyclically by the tsetse fly (Diptera: Glossinidae). The mode of transmission of *T. vivax* is distinct in that it can also be transmitted mechanically by biting flies, such as tabanids. This has resulted in the occurrence of AAT due to *T. vivax* outside the tsetse belts of Africa [6, 12]. Bovine trypanosomosis is a threat to livestock health and agricultural production and thereby affects rural development and poverty alleviation efforts in Africa [29].

Amongst the five *Theileria* species and subspecies that are known to exist in Zambia, the most economically important are *Th. parva parva* and *Th. parva lawrencei* [15]. *B. bovis* and *B. bigemina* are recognized as being of economic importance to the Zambian cattle sector [15]. *B. bigemina* is shown to be widespread in Zambia, whereas *B. bovis* is mostly restricted to the eastern and north-eastern parts of the country [10]. Infections caused by *B. bigemina* are more extensive than those caused by *B. bovis*, and this may be attributed to the wider vector range of *B. bigemina* [15].

In Zambia, trypanosomosis has more of an endemic na-

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ture and is characterized by high morbidity and rather low mortality. However, the devastating effects of trypanosomosis on livestock production are very obvious in areas where susceptible livestock are introduced in a tsetse-infested zone or *vice versa*. A good example is the plateau of eastern Zambia where devastating livestock trypanosomosis epidemics occurred after game and tsetse spread south and east from the Luangwa valley onto the plateau [29]. The reason for the epidemic nature of trypanosomosis is attributed to the diversity in trypanosome populations [16]. As a result of drug use and the high mortality rates in untreated animals infected with virulent strains, the likelihood that virulent strains will persist in the livestock that are susceptible to the domestic transmission cycle is low. On the low plateau of eastern Zambia, for example, only 20% of the trypanosome strains isolated from cattle had a virulent phenotype [17]. Moreover, laboratory experiments indicated that the presence of an infection with a low-virulence trypanosome strain confers protection against the deleterious effects of infection with a virulent strain [16].

This study aims to assess the prevalence of trypanosomes and selected piroplasms that are of great importance to livestock farming in the areas of Chama, Monze and Mumbwa in Zambia. The present study will generate information that will provide a glimpse of the nature of the behavior and diseases associated with these parasites among the cattle and goats in these areas.

MATERIALS AND METHODS

The study area and sample size: Sample collection was conducted in Chama (Muchinga province), Mumbwa (Central province) and Monze (Southern province) districts during the dry season (May to October) of 2010 (Fig. 1). A total of 472 heads of cattle from Chama (n=292), Mumbwa (n=96) and Monze (n=84) and 53 goats (all from Chama) were examined for the presence of animal African trypanosomes (*Trypanozoon*, *T. congolense* and *T. vivax*), *B. bigemina* and *Th. parva* using PCRs.

Sample collection and DNA preparation: Blood samples were collected from cattle and goats whose owners gave their consent to participate in the survey. Permission was obtained according to the standards of animal experimentation in Obihiro University of Agriculture and Veterinary Medicine (Approval No. 28–47), and Department of Veterinary and Livestock Development, Zambia (Approval No. 212CO100011192). Approximately 5 m*l* of blood was drawn from the jugular vein of each animal using vacutainer tubes with EDTA-2Na (Terumo Co., Tokyo, Japan) and loaded into capillary tubes, and the packed cell volume (PCV) values were determined. These procedures were conducted in the field at the sampling site. In addition, the total DNA of each blood sample (1 m*l*) was isolated using a DNA isolation kit for mammalian blood (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's suggested protocol. The isolated DNA was stored at −30°C until use.

The detection of hemoparasites by PCR: This study used 2 different PCR techniques to detect and identify trypanosomes: (i) a KIN-PCR, which amplifies internal transcribed spacer 1 (ITS1), was used for *Trypanozoon* (*T. brucei brucei*, *T. b. rhodesiense*, *T. b. gambiense*, *T. evansi* and *T. equiperdum*) and *T. congolense*; and (ii) a TviCatL-PCR, which amplifies the highly conserved Cathepsin L-like gene among *T. vivax* isolates [5, 19]. A PCR that amplifies apical membrane antigen-1 (AMA-1) was used for *B. bigemina,* while a p104 gene PCR, which uses IL755 and IL3231 primers to amplify the p104 gene, was used for *Th. parva* (Table 1) [24, 26]. All of the parasites were detected using single-step PCR methods; for the trypanosomes, the PCR reactions included 1 μl of 10 × reaction buffer, 0.3 μl 50 mM magnesium chloride, 250 μM dNTPs and 0.1 μl of *Taq* DNA polymerase (all from Thermo Fisher Scientific Inc., Waltham, MA, U.S.A.), 1 μl of each of the forward and reverse primers and 5.1 μl of double-distilled water; 0.5 μl of the DNA sample was added to the individual PCR mixtures. The PCRs were conducted using a Veriti™ Thermal cycler (Thermo Fisher Scientific). The PCR conditions have been described previously [13]. For the piroplasms, the PCR reactions included 1 μl of 10 × reaction buffer, 200 μM dNTPs and 0.1 μl of *Taq* DNA polymerase (AmpliTaq gold®) (all from Thermo Fisher Scientific), 0.5 μl each of the forward and reverse primers and 5.9 μl of double-distilled water. One microliter of the DNA sample was added to the individual PCR mixtures. The PCRs were conducted using a Veriti™ thermal cycler. The PCR conditions that were used for the *Babesia* and *Theileria* species have been described previously [24, 26].

Statistical analysis: The 95% confidence intervals were determined using the Creative Research Systems software program (accessed at www.surveysystems.com). The chi-squared and Fisher's exact tests were performed, the significance of their associations was tested, and *p*-values were determined using the GraphPad Prism software program (GraphPad Software Inc., La Jolla, CA, U.S.A.). Associations between the hematocrit values and the various parasitic infections were analyzed using a one-way analysis of variance, the strengths of the associations were tested using Tukey's mean difference, and *P*-values were determined using the GraphPad Prism software program.

RESULTS

Parasite prevalence by species: In the present study, 200 of the 525 (38.1%) samples were found to be positive for at least one hemoprotozoan parasite. *T. congolense*, *Trypanozoon*, *T. vivax*, *B. bigemina* and *Th. parva* were identified in cattle and goat samples collected from the three study locations (Table 2). *T. vivax,* which was detected in 19.8% of the samples, was the most common parasite. The prevalence of *T. vivax* in goats (37.7%) was significantly higher than that in cattle (17.8%) (Table 3). The second most common parasite was *B. bigemina,* which was present in 16.1% of cattle. This was followed by *T. congolense* (1.9% and 5.7% in cattle and goats, respectively), then *Trypanozoon* (1.0%in cattle only) and lastly *Th. parva* (0.6% in cattle only). *T. vivax* was the only parasite that had a significant difference in the infection rate between cattle and goats (Table 3). Twelve percent of

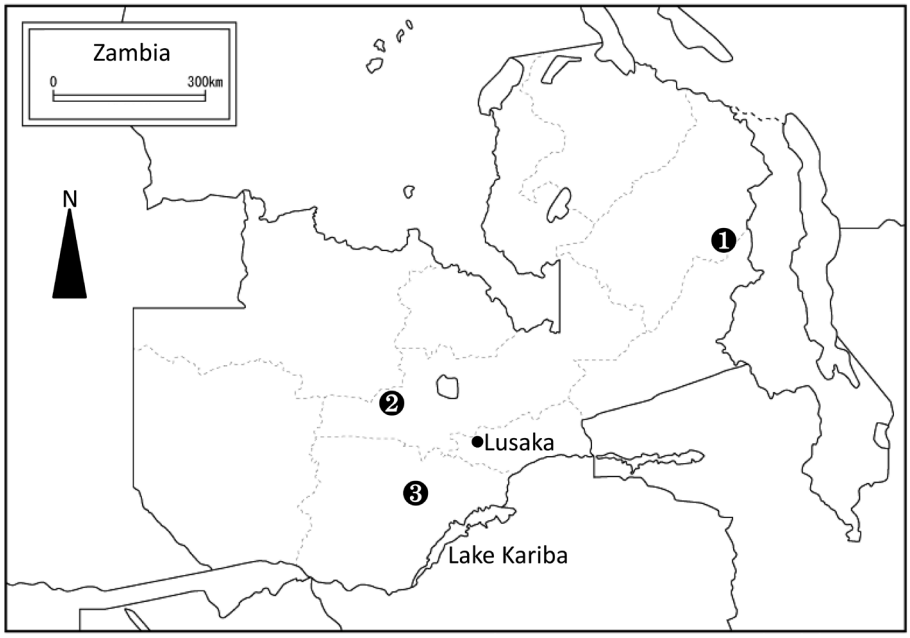

Fig. 1. A map of Zambia showing the sampled areas. The numbers on the map indicate the locations of Chama (❶), Mumbwa (❷) and Monze (❸). A free map of Zambia was obtained from http://www.freemap.jp.

Table 1. The PCR primer sets

Target gene	Primer	Sequence (5′→3′)	Specificity	Size (bp)	Reference
ITS1	KIN1	GCGTTCAAAGATTGGGCAAT	*T. congolense*	750[a)]	McLaughlin *et al.*, 1996
	KIN2	CGCCCGAAAGTTCACC	*Trypanozoon*	540[a)]	
CatL-like*	TviCatL1	GCCATCGCCAAGTACCTCGCCGA	*T. vivax*	177	Cortez *et al.,* 2009
	DTO 155	TTAAAGCTTCCACGAGTTCTTGATGATCCAGTA			
AMA-1	BI-AMA-F1	TACTGTGACGAGGACGGATC	*B. bigemina*	211	Sivakumar *et al.*, 2012a
	BI-AMA-R1	CCTCAAAAGCAGATTCGAGT			
P104	IL3231	ATTTAAGGAACCTGACGTGACTGC	*Th. parva*	496	Skilton *et al.*, 2002
	IL755	TAAGATGCCGACTATTAATGACACC			

**Trypanosoma vivax* cathepsin L-like cysteine protease. a) The KIN1 and KIN2 oligonucleotides are specific for both *T. congolense* (750 bp) and *Trypanozoon* (540 bp).

Table 2. The parasite infection rates at the three study locations

Location	Host	N	P	%	*Trypanozoon*		*T. congolense*		*T. vivax*		*B. bigemina*		*Th. parva*	
					P	%	P	%	P	%	P	%	P	%
Chama	Cattle	292	73	25.0	4	1.4	4	1.4	46	15.8	19	6.5	0	0
	Goats	53	23	43.4	0	0	3	5.7	20	37.7	0	0	0	0
Monze	Cattle	84	37	44.0	0	0	0	0	0	0	34	40.5	3	3.6
Mumbwa	Cattle	96	67	69.8	1	1.0	5	5.2	38	39.6	23	24.0	0	0
Total		525	200	38.1	5	1.0	12	2.3	104	19.8	76	14.5	3	0.6
P value					0.615		0.057		<0.0001*, a)		<0.0001*, b)		0.0004*, c)	

*Statistically significant ($P<0.05$). a) The *T. vivax* infection rate in Mumbwa was significantly higher than that at other locations. b) The *B. bigemina* infection rate in Monze was significantly higher than that at other locations. c) *Th. parva* was only found in Monze. N, number of samples; P, number of PCR-positive samples.

the infected samples were mixed infections (Table 4). The most frequent mixed infections were *T. vivax/B. bigemina* (7.0%), followed by *T. congolense/T. vivax* (2.5%), then *T. congolense/B. bigemina* (1.0%) and *B. bigemina/Th. parva* (1.0%) and lastly *T. congolense/T. vivax/B. bigemina* (0.6% in cattle) (Table 4).

Table 3. The infection rates for each species and parasite

Species	N	P	%	*Trypanozoon*		*T. congolense*		*T. vivax*		*B. bigemina*		*Th. parva*	
				P	%	P	%	P	%	P	%	P	%
Cattle	472	177	37.5	5	1.1	9	1.9	84	17.8	76	16.1	3	0.6
Goats	53	23	43.4	0	0	3	5.7	20	37.7	0	0	0	0
Total	525	200	38.1	5	1.0	12	2.3	104	19.8	76	14.5	3	0.6
P value				ND		0.111		0.025*, a)		ND		ND	

*Statistically significant ($P<0.05$). a) The *T. vivax* infection rate was significantly higher in goats than in cattle. ND, not done; N, number of samples; P, number of PCR-positive samples.

Table 4. Mixed infections according to the species

Species	TP	MI	%	Tc/Tv		Tc/Bb		Tv/Bb		Bb/Tp		Tc/Tv/Bb	
				P	%	P	%	P	%	P	%	P	%
Cattle	177	22	12.4	3	1.7	2	1.1	14	7.9	2	1.1	1	0.6
Goats	23	2	8.7	2	8.7	0	0	0	0	0	0	0	0
Total	200	24	12.0	5	2.5	2	1.0	14	7.0	2	1.0	1	0.5

Tc/Tv, Tc/Bb, Tv/Bb, Bb/Tp and Tc/Tv/Bb indicate a mixed infection with the corresponding parasites. TP, total number of PCR-positive samples; MI, number of mixed infection-positive samples; P, number of PCR-positive samples for each parasite. Tc, *T. congolense*; Tv, *T. vivax*; Bb, *B. bigemina*; Tp, *Th. parva.*

The prevalence rates according to location: No trypanosome-positive samples were found in the samples from Monze, and all the *Th. parva* positive samples were from Monze. The area with the highest cattle infection rate was Mumbwa (69.8%) followed by Monze (44.0%) and Chama (25.0%) (Table 2). Although there were no significant differences in the *Trypanozoon* and *T. congolense* infection rates, there were significant differences in the rates of *T. vivax, Th. parva* and *B. bigemina* positivity, with Mumbwa having a significantly higher prevalence of *T. vivax* (39.6%), and Monze having the only *Th. parva* positive samples and a significantly higher prevalence of *B. bigemina* (40.5%) (Table 2). Chama was the only area in which goat samples were collected; in the other study areas, only cattle samples were collected. *B. bigemina* was the only parasite found across all three areas, with the highest prevalence in Monze (40.5%) followed by Mumbwa (24.0%) and Chama (6.5%) (Table 2). In Chama and Mumbwa, *T. vivax* was the most prevalent trypanosome in cattle (15.8% and 39.6%, respectively) followed by *T. congolense* (1.4% and 5.2%) and finally *Trypanozoon* (1.4% and 1.0%) (Table 2).

Prevalence according to age and sex: There were no significant differences in the infection rates according to age or sex in the cattle and goats; however, male cattle (39.5%) had a higher rate of infection than female cattle (36.3%). In contrast, female goats (62.5%) had a higher rate of infection than male goats (38.5%). Adult animals (cattle, >2 years of age; and goats, >0.5 years of age) of both species had a higher incidence than juvenile animals (42.1 vs. 26.8% in cattle and 58.1 vs. 54.5% in goats).

The relationship between PCV and infection: According to the hematocrit values, there was no significant difference between the positive and negative samples from goats (data not shown). In cattle, however, there were significant differences between the mixed infections, the single infections and the negative samples, with the mixed infection samples having a significantly lower hematocrit value (Fig. 2).

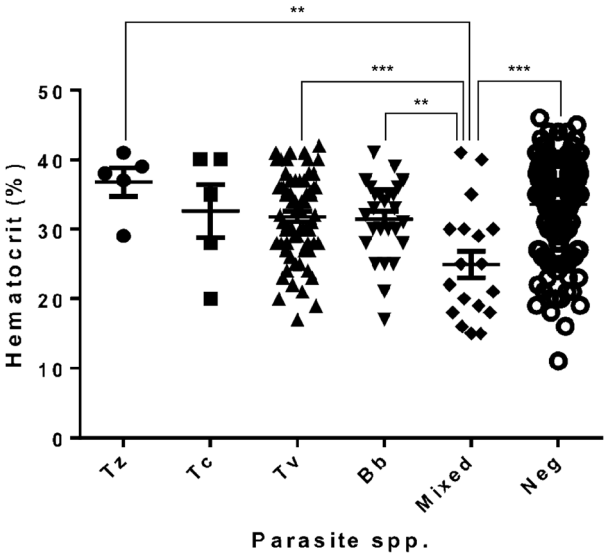

Fig. 2. A graph comparing the PCV values of cattle with various parasite infections. A comparison of the hematocrit values in cattle with mixed-infection, various parasitic infections and uninfected cattle. The bars and asterisks denote the levels of significance (**$P<0.05$, ***$P<0.0001$). Tz,*Trypanozoon;* Tc,*T. congolense*; Tv,*T. vivax*; Bb, *B. bigemina*; Mixed, mixed infections; Neg, negative for the screened parasites.

DISCUSSION

In this study, the prevalence of AAT and piroplasmosis

in the three districts of Chama, Monze and Mumbwa in Zambia was determined using PCR-based methods. The PCR-based screening results suggested that *T. vivax* was the most prevalent parasite in the study areas (Table 2). This can be attributed to the fact that this parasite, unlike other tsetse-transmitted trypanosomes, can also be transmitted mechanically by other biting flies—as has been shown in other studies outside the tsetse belts, especially in South America [6, 12]. The second most common parasite was *B. bigemina,* which was also the only parasite that was found in all three locations (Table 2). This is in line with the results of earlier studies which reported that babesiosis caused by *B. bigemina* is widespread throughout Zambia [10, 15]. Babesiosis caused by *B. bigemina* is widely distributed and is therefore suggested to be a potential threat to the country's cattle sector. This could be attributed to the diversity of *B. bigemina* vectors, which include *Boophilus microplus*, *Bo. decoloratus* and *Rhipicephalus evartsi*, and the fact that the principle host tick, *Bo. decoloratus,* is also widely distributed throughout the country [15, 21]. In addition, although babesiosis is recognized as an economically important disease, unlike theileriosis and trypanosomosis, its control and eradication have not been a priority. The control of babesiosis is mainly achieved through the treatment of sick animals; however, it should be noted that tick control through the use of acaricides, has also helped, even though it has mainly targeted theileriosis [15]. *Th. parva*, was the least prevalent species (<1%), and this prevalence is in agreement with the International Fund for Agricultural Development (IFAD) Zambia Country Programme evaluation report of 2014 [9]. This can be attributed to the various efforts in tick control and vaccination against East Coast Fever that have been carried out by the Government of the Republic of Zambia and other donor agencies [9, 15, 20]. The samples were collected from the districts of Chama, Monze and Mumbwa. Accordingly, no trypanosome-positive samples were found in samples obtained from Monze; this may be attributed to the eradication of tsetse flies from the areas around Lake Kariba (Table 2 and Fig. 1).

Twelve percent of the positive samples were mixed infections (Table 4); there were no significant differences among the host species. However, in cattle, there was a relationship between the hematocrit values and the presence of mixed infection (Fig. 2). Although there was no significance in the PCV values of the single infection and negative samples, there was a significant decrease in the PCV values of the mixed infection samples. This result suggested that mixed infection was responsible for more cases of clinical disease in cattle. The development of anemia is one of the most common signs of disease caused by hemoparasites in susceptible cattle. The level of anemia or PCV usually gives a reliable indication of the disease status and the productive performance of an infected animal [27, 28]. The measurement of the PCV of non-infected animals helps to form a baseline for the comparison of PCVs among the sampled animals, because the PCV value is affected by confounding factors other than parasitic infections [30]. While goats had a higher infection rate than cattle (Table 3), the PCV values of the infected and non-infected goats did not differ to a statistically significant extent. This could be because they are more tolerant to these parasites than cattle and because trypanosomosis in goats commonly follows a subclinical path—usually with low but persistent parasitemia [3, 8, 22, 23]. Our results showed that goats can be an important reservoir for trypanosomes and that the treatment of goats could lead to a reduction in the incidence of Trypanosomosis in Zambia and other African countries.

In conclusion, we revealed that the prevalence of hemoprotozoan parasites was high in the cattle and goats in Zambia. This result suggests that the control of these parasites and vectors is important for the improvement of the agricultural sector in Zambia.

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