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Widespread occurrence of *Trypanosoma vivax* in bovines of tsetse- as well as non-tsetse-infested regions of Ethiopia: A reason for concern?

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ABSTRACT

A cross-sectional study was undertaken to assess the prevalence of bovine trypanosomosis in some tsetse-infested and tsetse-free areas of Ethiopia. From August 2010 till April 2011, a total of 1524 animals were parasitologically examined and compared by the haematocrit centrifugation technique (Woo test) and polymerase chain reaction (ITS-1 PCR). The ITS-1 PCR was more sensitive and more accurate in species identification than the Woo test.

In ITS-1 PCR, an overall trypanosome prevalence of 31.0% was observed that is significantly ($P < 0.001$) higher than in the Woo test (5.3%). *Trypanosoma vivax* was the predominant taxon (24.9%), followed by *T. theileri* (6.0%), *T. congolense* (2.9%) and *Trypanozoon* (1.6%). Mixed infections were quite common (14% of all infections). The overall prevalence of trypanosome infections in tsetse area (32.4%) was not different from non-tsetse area (30.5%) neither were the prevalences of *T. vivax* in both areas (respectively 22.6% and 25.7%).

With these high prevalences, bovine trypanosomosis continues to hinder animal production and productivity in Ethiopia, both in tsetse-infested and non-infested parts of the country. Attempts to control African trypanosomosis should also pay attention to mechanically transmitted pathogenic trypanosomes and should adopt the most advanced molecular tests for species identification.

1. Introduction

African bovine trypanosomosis (nagana) occurs in vast areas of sub-Saharan Africa with a devastating impact on livestock productivity thus directly contributing to hunger, poverty, malnutrition and suffering of entire communities.[1] Nagana is caused by *Trypanosoma* (*T.*) *congolense*, *T. vivax* and *T. brucei* spp., all belonging to the *Salivaria* section. It affects all domestic animals and the symptoms are fever, listlessness, emaciation, hair loss, conjunctivitis, oedema, anaemia, and paralysis. Clinical manifestations of nagana are not pathognomonic and are determined by the host, as well as by the trypanosome species. Animals that survive often remain infected for several months or years, exhibiting fluctuating parasitaemia and thus serve as disease reservoir. Cattle are also commonly infected by the ubiquitous *T. theileri*, which is a non-pathogenic species of the *Stercoraria* section (Hoare, 1972).

T. brucei, *T. congolense* and *T. vivax* are transmitted by tsetse flies, heamatophagous flies of the genus *Glossina*. Under natural conditions, *T. vivax* can also be transmitted

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[1] http://www.africa-union.org/Structure_of_the_Commission/depPattec.htm.

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mechanically by biting flies such as horse flies (*Tabanidae*) and stable flies (*Stomoxys*) (Mihok et al., 1995; Oluwafemi et al., 2007; Sinshaw et al., 2006). This type of transmission has caused tsetse-independent spread of *T. vivax* in Africa and Latin America (Abebe and Jobre, 1996; Davila and Silva, 2000).

Owing to the low specificity of clinical manifestations, diagnosis of bovine trypanosomosis cannot be exclusively based on clinical signs. Confirmation of the infection is only possible by microscopic examination of the blood to demonstrate trypanosomes.

In Ethiopia, quite a number of epidemiological studies were carried out on bovine trypanosomosis using conventional parasitological techniques such as dark field buffy coat, thin and thick blood smear as well as serological tests (Bitew et al., 2011; Cherenet et al., 2004, 2006; Mihret and Mamo, 2007; Sinshaw et al., 2006). However, during chronic and early infections, parasitaemia can be very low making it difficult to detect and to correctly identify the parasites with these conventional parasitological techniques leading to underestimation of the prevalence and incorrect species identification (Büscher, 2001). Thus, the information on prevailing species of trypanosomes and their occurrence in animals in Ethiopia is inaccurate and fragmented. However, gathering accurate epidemiological data is crucial for developing treatment and control strategies to protect livestock against trypanosomosis. With the use of different primer sets, it is possible to distinguish different trypanosome species in the same specimen, as was shown in a study assessed the differential distribution of pathogenic African trypanosome species in Ethiopian cattle (Cherenet et al., 2006). However, this can also be achieved in a PCR with one single primer set targeting the ribosomal 18S gene (Geysen et al., 2003) or the ribosomal internal transcribed spacer (ITS) region (Claes et al., 2003; Desquesnes et al., 2001). The ITS of the ribosomal RNA locus of eukaryotes varies both in sequence and length between taxa but is highly conserved within a given taxon.

This study was undertaken to assess the differences in prevalences of bovine trypanosomosis between tsetse-infested and tsetse-free areas covering a wide range of study sites of Ethiopia and making use of an accurate yet simple molecular test for species identification.

2. Materials and methods

2.1. Study area

In Ethiopia, the south western part following the major river basins is the major tsetse infested area (Fig. 1). The rest of the country, the north, south east and east is considered tsetse free (Sinshaw et al., 2006; Wint et al., 2010). Six study zones were selected based on previous history of tsetse presence or absence. Jimma and Gurage, in south-western Ethiopia, were selected as tsetse infested area *where Glossina morsitans submorsitans* and *G. pallidipes* were reported (Leak and Mulatu, 1993; Negash et al., 2012). Horro-Guduru is also located in the west of the country but with an altitude of 2241–2430 m above sea level (m.a.s.l.). It is considered as tsetse free highland. West Gojjam, South

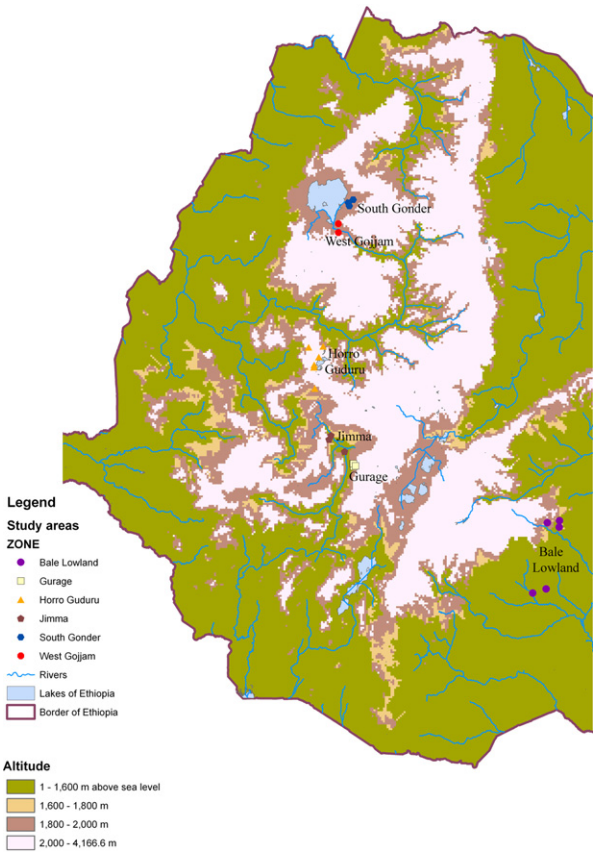

Fig. 1. Map of the study sites with their altitude above sea level.

Gonder and Bale Lowland zones are located, respectively, in the north and the east of the country and are considered tsetse free (Sinshaw et al., 2006). These considerations were corroborated by projecting the coordinates of the study zones on a map visualising the predicted distribution of tsetse species in Africa (Fig. 2). This map was constructed using ArcGIS 9.2 (ESRI, Redlands, California) by combining the raster files on the predicted distribution of different tsetse species,[2] clipping the resulting map with a file of the boundaries of Ethiopia and converting the selected area of the raster map into a vector file. Within each study zone, peasant associations (PAs) were selected for data collection and blood sampling from their cattle.

2.2. Cross-sectional study

At 95% confidence interval, a sample size of 1258 animals is required, assuming that the real prevalence equals 20% in Gurage, Jimma, South Gonder and West Gojjam, whereas 50% prevalence is considered in other zones since there is no previous report (Thrusfield, 2005). In addition to sampling blood for parasitological and molecular diagnosis, data were collected *via* a questionnaire. In this questionnaire, the number of livestock kept by the farmer,

[2] http://www.fao.org/ag/againfo/programmes/en/paat/maps.html.

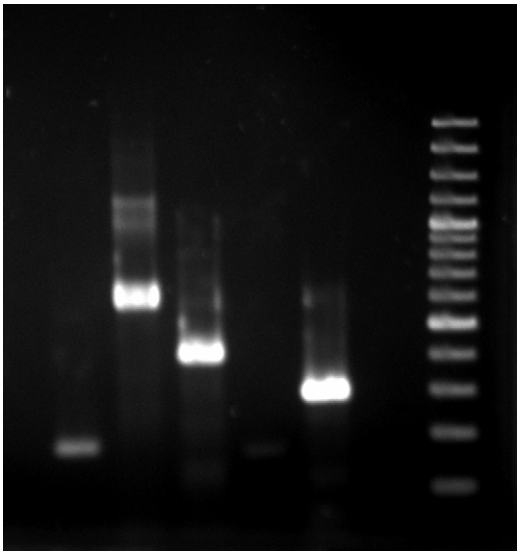

Fig. 2. Amplicons with different lengths generated in ITS1-PCR according to the species. *T. vivax*: lanes 1 and 4, *T. congolense Savannah type*: lane 2, *Trypanozoon*: lane 3, *T. theileri*: lane 5, negative control: lane 6, 100 bp DNA ladder: lane 7.

the current health status of the animal, anti-nagana treatment history and response to treatment and commonly used drugs were recorded.

2.3. Animals

Local zebu and Borana breed were randomly selected irrespective of age, sex and body condition. Age and body condition of the animals were determined based on response from the owners and observation during the survey. Animals that had received trypanocidal therapy within one week before the sampling were excluded. All animals were managed under extensive production system where they were allowed to freely graze during the day. The farmers in the study sites practice a mixed farming system. Animals play a role in providing draft power to plough the land, milk and milk products and manure for crop-fields. Other common livestock reared are sheep, goats, equines and, in Bale Lowlands, dromedary camels.

2.4. Blood collection

From each bovine, 9 mL of jugular vein blood were collected into heparinised Venosafe tubes (Terumo) for further analyses.

2.5. Haematocrit centrifugation technique (Woo)

The haematocrit centrifugation technique was performed as described by Woo (1970). Briefly, volumes of about 50 μL of blood were collected from the Venosafe tube into two microhaematocrit capillary tubes. The tubes were sealed and centrifuged at 15,000 rpm for 5 min to concentrate trypanosomes in the buffy coat layer. After reading packed cell volume (PCV), the capillaries were placed in a Woo viewing chamber. A cover slip of 24 mm × 24 mm was placed on the capillaries, where after the space between the tubes and the cover slip was filled with water to reduce light diffraction. The buffy-coat plasma junction was examined for presence of trypanosomes, and species identification was assessed based on morphology and motility.

2.6. Storage of blood and DNA extraction

For DNA extraction and PCR, 800 μL of blood from the Venosafe tube were mixed with an equal volume of AS1 blood lysis buffer (Qiagen) and preserved at ambient temperature. DNA was extracted with the QIAamp mini blood kit from 200 μL blood/AS1 buffer into 200 μL elution buffer according to the manufacturer's instructions. Extracted DNA was stored at −20 °C.

2.7. Polymerase chain reaction (PCR)

Species-specific trypanosome DNA was amplified by an Internal Transcribed Spacer 1 (ITS-1) PCR adapted from Desquesnes et al. (2001). PCR conditions were: 25 μL reaction volume contained 2.5 mM $MgCl_2$ (Qiagen), 200 μM of each dNTP (Eurogentec), 0.8 μM of ITS-1 forward primer (5′-TGTAGGTGAACCTGCAGCTGGATC-3′) and 0.8 μM of ITS-1 reverse primer (5′-CCAAGTCATCCATCGCGACACGTT-3′) (Biolegio), 0.5 U Hotstar Taq polymerase (Qiagen) 0.1 mg/mL acetylated BSA (Promega) in H_2O and 2.5 μL target DNA corresponding to 1.25 μL of blood. Cycling conditions were: 94 °C for 15 min, 40 cycles of 30 s at 94 °C followed by 30 s at 60 °C and 30 s at 72 °C, final extension at 72 °C for 5 min. Interpretation of the results, after electrophoresis in 2% agarose gels and staining with Ethidium Bromide (EtBr), is based on the characteristic amplicon lengths: 150 bp, 350 bp, 450 bp, 650 bp for respectively, *T. vivax*, *T. theileiri*, *Trypanozoon* and *T. congolense Savannah type* (Fig. 2). On specimens negative in ITS-1 PCR, a vertebrate cytochrome B specific PCR was carried out to assess presence of amplifiable DNA and absence of PCR inhibitors in the test specimen (Kocher et al., 1989). PCR conditions were as follows: 25 μL reaction volume contained 2.5 mM $MgCl_2$ (Qiagen), 200 μM of each dNTP (Eurogentec), 0.8 μM of L14841 forward primer (5′-CCATCCAACATCTCAGCATGATGAAA-3′) and 0.8 μM of H15149 reverse primer (5′-GCCCCTCAGAATGATATTTGTCCTCA-3′) (Biolegio), 0.5 U Hotstar Taq polymerase (Qiagen) 0.1 mg/mL acetylated BSA (Promega) in H_2O and 2.5 μL target DNA. Cycling conditions were: 94 °C for 15 min, 40 cycles of 30 s at 94 °C followed by 30 s at 52 °C and 30 s at 72 °C, final extension at 72 °C for 5 min. The amplicon is 400 bp long.

2.8. Data analysis

All data were recorded in Microsoft Excel. Statistical Package for Social Science (SPSS) version 19 was used for statistical analysis. Chi-square (X^2) test was applied for assessing significance of differences with 95% confidence interval.

Table 1
Peasant association (PA) with geographical coordinates, altitude (m.a.s.l.) and number of animals sampled (*N*).

Zone	District	PA	m.a.s.l.	Coordinates	*N*
Gurage	Enimore and Ener	Wadesa	1718	N07°56′77, E37°42′75	38
		Gomosha	1927	N07°59′61, E37°42′41	48
		Shumoro	1920	N07°59′61, E37°42′49	63
		Wojira	1984	N07°57′54, E37°44′46	50
Jimma	Chora Botor	Yatu	1466	N08°25′19, E37°22′39	75
		Keta bosso	1600	N08°21′57, E37°20′53	76
	Sokoru	Abelti	1678	N08°10′49, E37°34′57	61
Horro Guduru	Horro	Didbe Kistana	2396	N09°36′14, E37°11′16	81
		Bona Abona	2430	N09°45′03, E37°01′97	49
		Gida Dasho	2328	N09°26′90, E37°06′79	25
		Nashe Igu	2248	N09°45′86, E37°15′20	89
	Jimma- Genati	Gudetu Genati	2269	N09°29′09, E37°06′99	37
		Kelala Dimtu	2241	N09°07′65, E37°07′61	102
Bale Lowland	Goro	Ro'o-nagaya	1681	N06°02′42, E40°25′55	109
		Melka Butta	1570	N06°06′08, E40°37′18	80
	Ginnir	Tullicha	1386	N07°02′64, E40°49′09	74
		Doyyo	1966	N07°06′40, E40°38′23	71
		Keteti	1425	N07°08′30, E40°49′29	39
South Gonder	Fogera	Kokit	1793	N11°58′78, E37°42′39	72
		Shina	1792	N11°53′04, E37°38′89	67
		Shaga	1790	N11°56′10, E37°38′29	73
West Gojjam	Bahir Dar Zuria	Meshenti	1700	N11°29′21, E37°29′39	72
		Zenzelima	1907	N11°37′62, E37°29′93	73

3. Results

The cross-sectional study was carried out from August 2010 to April 2011. A total of 1524 whole blood samples were collected (Table 1).

3.1. Woo test

Results of the Woo test are represented in Table 2. An overall prevalence of trypanosome infection of 5.3% (CI 4.2–6.4%) was observed with *T. vivax* as the significantly ($P < 0.0001$) predominant taxon (4.3%, CI 3.3–5.3%) followed by *T. congolense* (1.5%, CI 0.09–2.1%) and *Trypanozoon* (0.3%, CI 0.03–0.57%). Regarding *Trypanozoon*, it is impossible to distinguish *T. brucei* from *T. evansi* morphologically in the Woo test. Of all infections, 14.8% (CI 13–17.6%) were recognised as co-infection. Only two *T. congolense* infections (mixed with *T. vivax*) were observed in tsetse free areas. *T. vivax* was significantly ($P < 0.001$) more prevalent in tsetse (7.8%, CI 6.5–9.1%) than in non-tsetse area (3.0%, CI 2.1–3.9%). Overall prevalence was significantly higher ($P < 0.001$) in tsetse (10.9%, CI 9.3–12.5%) than in non-tsetse area (3.2%, CI 2.3–4.1%).

3.2. ITS-1 PCR

Results obtained in ITS-1 PCR are represented in Table 3 and Fig. 3. The overall prevalence was 31.0% (CI 28.7–33.3%) what is significantly higher than in Woo ($P < 0.001$). Also in PCR, *T. vivax* was significantly the predominant taxon (24.9%, CI 22.7–27.1%), followed by *T. theileri* (6.0%, CI 4.8–7.2%), that was not observed in Woo, *T. congolense Savannah type* (2.9%, CI 3.7–2.1%) and *Trypanozoon* (1.6% CI 0.97–2.2%). As in the Woo test, co-infections represented about 15% (CI 13.2–16.8%) of the observed infections and co-infections with all three taxa were not observed. No

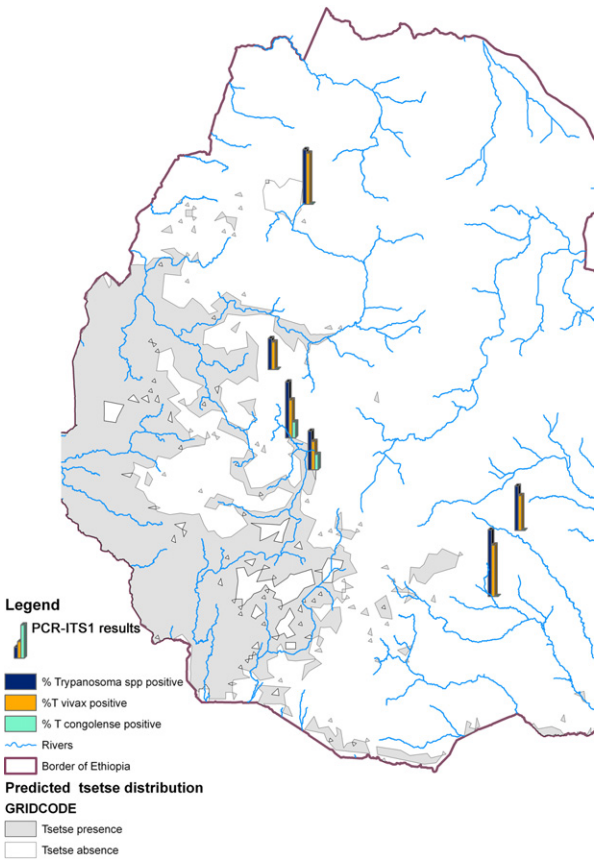

Fig. 3. Percentage of animals positive in the ITS-1 PCR for trypanosomes, *T. vivax* or *T. congolense* and the predicted tsetse distribution in Ethiopia.

Table 2
Number of animals found positive for trypanosomes in the Woo test and the putative identification of the trypanosome taxon in the specimen, grouped by zone and tsetse status.

Zone	Tsetse area	Tc			Tv		Tz		Tc + Tv		Tv + Tz	
		N	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Gurage	Yes	199	6	3.0	1	0.5	0	0	1	0.5	1	0.5
Jimma	Yes	212	7	3.3	21	9.9	0		7	3.3	1	0.5
Horro-Guduru	No	383	0	0	11	2.9	0	0	2	0.5	0	0
Bale Lowland	No	373	0	0	15	4.0	3	0.8	0	0	0	0
South Gonder	No	212	0	0	2	0.9	0	0	0	0	0	0
West Gojjam	No	145	0	0	3	2.1	0	0	0	0	0	0
Total		1524	13	0.9	53	3.5	3	0.2	10	0.7	2	0.1

N, total number of animals examined; *n*, number of animals found positive; %, prevalence; Tc, *T. congolense*; Tv, *T. vivax*; Tz, *Trypanozoon*.

Table 3
Number of animals found positive for trypanosomes in the ITS-1 PCR according to taxon identification, grouped by zone and tsetse status.

Zone	Tsetse area	Tc			Tv		Tt		Tz		Tc + Tv		Tc + Tt		Tc + Tz		Tv + Tt		Tv + Tz		Tt + Tz	
		N	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Gurage	Yes	199	13	6.5	29	14.6	0	0	0	0	7	3.5	0	0	1	0.5	2	1.0	0	0	0	0
Jimma	Yes	212	13	6.1	42	19.8	5	2.4	5	2.4	7	3.3	2	0.9	1	0.5	4	1.9	2	0.9	0	0
Horro-Guduru	No	383	0	0	63	16.4	5	1.3	4	1	0	0	0	0	0	0	8	2.1	1	0.3	0	0
Bale Lowland	No	373	0	0	68	18.2	20	0.5	2	0.5	0	0	0	0	0	0	18	4.8	3	0.8	2	0
South Gonder	No	212	0	0	67	31.6	4	1.9	1	0.5	0	0	0	0	0	0	7	3.3	0	0	0	0
West Gojjam	No	145	0	0	49	33.8	12	8.3	2	1.4	0	0	0	0	0	0	2	1.4	0	0	1	0.7
Total		1524	26	1.7	318	20.9	46	3	14	0.9	14	0.9	2	0.1	2	0.1	41	2.7	6	0.4	3	0.2

N, total number of animals examined; *n*, number of animals found positive; %, prevalence, Tc, *T. congolense*, Tv, *T. vivax*, Tz, *Trypanozoon*, Tt, *T. theileri.*

T. congolense was detectable in specimens from tsetse-free areas. Overall prevalence of trypanosome infections in tsetse area (32.4%, CI 30.1–34.7%) was not different from non-tsetse area (30.5%, CI 28.2–32.8%) neither were the prevalences of *T. vivax* in both areas (respectively 22.6%, CI 20.5–24.7% and 25.7%, CI 23.5–27.9%).

3.3. Effect of treatment on trypanosome infection prevalence

Regarding results obtained in ITS-1 PCR, no significant differences were observed in overall trypanosome infection between animals that were treated with anthelminthics (34.9% CI 32.5–37.3%), trypanocides (33.6% CI 31.2–36.0%), antibiotics (25% CI 22.8–27.2%) and without treatment (29.7% CI 27.4–32%). The trypanocidal drugs in use were diminazene aceturate and isometamidium chloride.

3.4. Age, sex, body condition, packed cell volume

Regarding trypanosome infections, no significant differences ($P > 0.05$) were observed between animals of different age groups (calves less than 1.5 years, young heifers and bulls between 1.5 and 4 years, old animals older than 4 years), sex and body conditions (good/poor). However, PCV tends to be lower in animals with trypanosome infection than in non-infected animals (Table 4). In particular, animals with *T. congolense* infection, single or mixed, have a significantly lower PCV than non-infected animals, both observed in Woo and in ITS-1 PCR.

Table 4
Mean PCV and standard deviation (SD) of animals according to infection status as assessed in ITS-1 PCR and Woo.

Infection status	ITS-1 PCR		Woo	
	Mean	SD	Mean	SD
Non-infected	28.0	4.4	27.8	4.6
Tc	21.5*	4.2	24.1*	5.3
Tc + Tt	22.5*	3.5		
Tc + Tv	20.4*	4.4	20.8*	6.3
Tc + Tz	22.5*	3.5		
Tt	28.3	4.7		
Tt + Tz	27.7	4.9		
Tv	27.24	5.1	24.8	6.1
Tv + Tt	27.0	5.5		
Tv + Tz	26.7	5.3	30.0	4.2
Tz	25.9	5.2	25.0	5.0

Tc, *T. congolense*; Tv, *T. vivax*; Tz, *Trypanozoon*; Tt, *T. theileri.*
* Significant difference with non-infected animals.

4. Discussion

This study was undertaken to assess the overall species-specific prevalence of bovine trypanosomosis in some tsetse-infested and tsetse-free areas of Ethiopia. Use was made of the parasitological Woo test and the molecular ITS-1 PCR test, the latter being putatively more sensitive and accurate for species identification.

The choice of these tests was based on sensitivity and ease of application. In the Woo test, capillary tubes are centrifuged and the buffy coat examined directly under the microscope at low magnification (10×10). The species is identified by morphology and characteristic movement of the trypanosome. Two capillaries per animal can be processed within 15 min. Alternative methods such as

dark field and anion exchange centrifugation might be respectively more accurate in species identification or sensitivity but are more complex and need more sophisticated microscopes (Murray et al., 1977; Paris et al., 1982). Among molecular tests, ITS1-PCR, allows identifying the trypanosome taxon in one single PCR. Since this PCR targets a 100–200 copy number of a ribosomal DNA sequence, the sensitivity is high (Desquesnes et al., 2001; Njiru et al., 2005) and the test is less complex than PCR-Restriction Fragment Length Polymorphism or Fluorescent Fragment Length Barcoding (Geysen et al., 2003; Hamilton et al., 2008).

The higher sensitivity of the ITS1-PCR is confirmed by our data. With ITS1-PCR, the observed overall prevalence was about five times higher than with the Woo. Our data also support the assumption that molecular diagnostics in general and ITS1-PCR in particular are more accurate for taxon identification than the Woo technique. Indeed, only in ITS1-PCR we observed infections with the non-pathogenic *T. theileri*. Furthermore, in the Woo test we diagnosed two animals with *T. congolense* in non-tsetse infested areas, while this species depends on tsetse for its natural transmission. This might be explained by cattle imported from tsetse-infested areas (Cherenet et al., 2006). However, in ITS1-PCR, these two animals were found negative for *T. congolense* but positive for *T. vivax* which is more consistent with the observed absence of *T. congolense* around lake Tana, a tsetse-free area in Ethiopia (Sinshaw et al., 2006).

The higher sensitivity of the ITS1-PCR allows a better interpretation of the epidemiological situation. Using the Woo test, trypanosomosis prevalence in tsetse-infested area, was significantly higher (10.9%) than in non-tsetse area (3.2%) while the molecular test showed no differences between both areas (about 30%). Hence, for epidemiological purposes, ITS1-PCR may be an additional tool to classical parasitological tests.

In both tsetse and non-tsetse areas, *T. vivax* was the predominant species. For tsetse infested regions, these results are in line with other studies in Ethiopia and Western Kenya (Kebede and Animut, 2009; Thumbi et al., 2010). The relative predominance of species should however be interpreted with caution since both microscopy and molecular diagnostic tests cannot detect infections with parasitemia below their detection limit. This particularly applies to *Trypanozoon* taxa (*T. brucei* and *T. evansi*) known for usually low parasitemia. It is thus not surprising that in other studies, *T. brucei* remained undetectable in bovine in Amhara, Ethiopia (Cherenet et al., 2006). In our study, the presence of *Trypanozoon* in tsetse and non-tsetse areas is confirmed but the actual prevalence may be considerably higher than observed here (1.6% with ITS1-PCR).

The advantage of molecular diagnostics for assessing prevalence is further illustrated by our data suggesting that, according to Woo, the treatment with anthelminthics would protect against trypanosomosis (0/129 infected) in contrast with what is observed with ITS1-PCR (45/129). Whether the differences observed with the two tests could be explained by an effect of anthelmintics on parasitaemia level is worth investigating. That the reported trypanocides have no effect on observed trypanosomosis prevalence, is striking. Although the study did not intend to collect high quality data on the trypanocidal use, our data suggest that transmission is very high or that treatment is not efficient which could be explained by underdosing, fake drugs or drug resistance. For twelve *T. congolense* strains that were isolated during the field work in Gurage and Jimma, it was proven *in vivo* that they are resistant against diminazene aceturate and isometamidium chloride (Moti et al., in press). For *T. vivax*, data on drug sensitivity are currently missing.

Infection with trypanosomes seems unrelated to age, sex and general condition of the animals, in concordance with the findings of other authors (Bitew et al., 2011; Dagnachew et al., 2005; Kebede and Animut, 2009). That trypanosomosis is not necessarily associated with poor body condition or emaciation, as is the case in this study, can be explained in situations where nutrient supply is not a limiting factor (Holmes et al., 2000). Only infection or co-infection with *T. congolense* was significantly associated with reduced PCV, in line with many earlier observations (Abebayehu and Biniam, 2010; Dagnachew et al., 2005; Holmes et al., 2000; Masumu et al., 2006; Mihret and Mamo, 2007; Miruk et al., 2008; Pathak, 2009) but in contrast with the report of Cherenet et al. (2006). As a result, anaemia is not a reliable parameter for infection with African trypanosomes in general.

Observed prevalences in this study are difficult to compare with other studies conducted in Ethiopia due to variations in diagnostic techniques, geographical situation, season, biting fly densities, treatment and tsetse control activities (Abebayehu and Biniam, 2010; Bitew et al., 2011; Dagnachew et al., 2011; Kebede and Animut, 2009; Mihret and Mamo, 2007; Miruk et al., 2008; Mulaw et al., 2011; Rowlands et al., 1993; Sinshaw et al., 2006). Although, in this study, we did not investigate the actual presence of tsetse flies in the study regions, we consider that the high prevalences of *T. vivax* in tsetse infested (22.6%) but particularly in allegedly non-tsetse infested areas (25.7%) is a matter of concern. This study did also not investigate commercial exchange of animals between tsetse infested and non-infested zones. Thus, although we cannot exclude that some of the *T. vivax* positive animals were imported from tsetse-infested areas, most likely there is local transmission in non-tsetse areas by blood sucking flies. For *T. vivax*, it is well established that this species can adapt to a non-tsetse dependent transmission cycle (Davila and Silva, 2000). As a consequence, the eradication of tsetse flies in Ethiopia, being an objective of the Pan African Tsetse and Trypanosomosis Eradication Campaign (PATTEC) will have little effect on the prevalence of *T. vivax*, even if tsetse-transmitted *T. vivax* strains might genetically be different from non-tsetse-transmitted strains and cannot readily adapt to transmission by other blood sucking flies. Within this regard, *T. vivax* isolates of this study from tsetse-infested and tsetse-non-infested areas will be geno- and phenotyped.

5. Conclusion

With prevalences around 30%, both in tsetse infested and non-infested parts of the country, bovine

trypanosomosis continues to hinder animal production and productivity in Ethiopia. Molecular diagnostics identified *T. vivax* as the predominant pathogenic trypanosome affecting cattle in the studied regions. Therefore, attempts to control African trypanosomosis should also pay attention to mechanically transmitted pathogenic trypanosomes and should adopt the most advanced molecular tests for species identification.

Conflict of interest statement

None of the authors has any financial or personal relationship that could inappropriately influence or bias the content of the paper.

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