Bovine trypanosome species prevalence and farmers' trypanosomiasis control methods in south-western Uganda

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A cross-sectional study was conducted in Mbarara district, south-western Uganda in May 2012 to determine the burden of African animal trypanosomosis (AAT) in the semi-intensive dairy production systems where pyrethroid acaricides are frequently used in the control of tick-borne diseases (TBDs). A total of 295 cattle blood samples were taken and analysed using a single pair of primers previously designed to amplify internal transcribed spacer (ITS1) of trypanosome ribosomal deoxyribonucleic acid (rDNA). A structured questionnaire was administered to 55 participating livestock farmers to generate data on acaricide and trypanocidal drug usage. The overall prevalence of trypanosome species was 2.4% (95% CI; 1.0% – 4.8%); *Trypanosoma vivax* was the most predominant species (2.0%; 95% CI; 0.7% – 4.4%). A single mixed infection of *T. vivax* and *Trypanosoma brucei s.l.* was detected. All the participating farmers used acaricides for tsetse and TBD control; 89.1% of the acaricides used were pyrethroids. About half of the farmers used trypanocidal drugs, mainly diminazene formulations (Berenil®). Low prevalence of trypanosomes in examined samples is most likely related to the frequent use of pyrethroid insecticides, trypanocides and restricted grazing (paddocking and tethering). These rigorous management practices are geared towards optimising production of exotic dairy breeds kept in this region that are highly susceptible to TBDs and AAT.

Introduction

African animal trypanosomosis (AAT) affects all domestic animals, as well as a wide range of wildlife species that serve as a reservoir of infection for both humans and domestic animals (Anderson *et al.* 2011). In cattle, the disease is caused by *Trypanosoma vivax*, *Trypanosoma congolense* and *Trypanosoma brucei brucei* (Clarkson 1976; Magona, Walubengo & Odimim 2008). Trypanosomes are transmitted cyclically by the tsetse fly (Diptera: Glossinidae), except *T. vivax*, which can also be transmitted mechanically by tsetse flies and other biting flies such as tabanids. This has resulted in AAT as a result of *T. vivax* occurring outside of the tsetse belts of Africa (Desquesnes & Dia 2003; Kone *et al.* 2011).

Bovine trypanosomosis is a threat to livestock health and agricultural production, thereby affecting rural development and poverty alleviation in Africa (Machila *et al.* 2003; Swallow 1998, 2000). In Uganda, the disease constrains livestock production and significantly prevents livestock-crop integration (Magona *et al.* 2005). About 2.2 million heads of cattle countrywide are at risk of AAT (Magona *et al.* 2005). In south-western Uganda, and particularly Kashaari county, Mbarara district, the majority of farmers keep exotic dairy cattle breeds (mainly Ankole long-horned and Friesian hybrids), which are highly susceptible to tick-borne diseases (TBDs) and therefore require a high level of tick control (Muhanguzi, Ikwap, Picozzi & Waiswa 2010; Muhanguzi, Matovu & Waiswa 2010). Consequently, farmers have stepped up acaricide usage in order to maintain the health of their animals (Muhanguzi, Ikwap, Picozzi & Waiswa 2010; Muhanguzi, Matovu & Waiswa 2010). In addition, farmers in this region frequently use isometamidium chloride to control AAT – a practice believed to keep the prevalence of the disease low in the region (Waiswa & Katunguka-Rwakishaya 2004). There is, however, a lack of accurate data generated by highly sensitive and specific molecular techniques on the prevalence of AAT in the district to inform policy makers on AAT control. Similarly, there is little accurate current information on acaricide, insecticide and trypanocidal drug usage in the control of tsetse and tick-borne diseases (TTBDs). The information available about AAT burden in south-western Uganda, for example, was generated about a decade ago by parasitological techniques (Waiswa & Katunguka-Rwakishaya 2004) that have been reported previously to be less sensitive than molecular techniques (Picozzi *et al.* 2002). The current study was therefore carried out to establish the actual burden of AAT in south-western Uganda and the rate at which acaricides, insecticides and trypanocides are used in the control of TTBDs. This information is very important in guiding and prioritising integrated control of TTBDs, which is important in improving the dairy sector in the region and integrating livestock-crop production.

Materials and methods

Study area

To study the actual burden of AAT in south-western Uganda and the rate at which acaricides and trypanocidal drugs are used for TBD and AAT control respectively, a cross-sectional study was carried out in Kashaari county, Mbarara district, in May 2012. The geography, vegetation and climate of the study area have been described previously (Muhanguzi, Ikwap, Picozzi & Waiswa 2010; Muhanguzi, Matovu & Waiswa 2010). The study area was selected because farmers in Kashaari county have predominantly changed from communal grazing to restricted (paddocking) semi-intensive cattle rearing (Muhanguzi, Ikwap, Picozzi & Waiswa 2010; Muhanguzi, Matovu & Waiswa 2010). Dairy cattle production is the mainstay of farmers' livelihoods in south-western Uganda and particularly in Kashaari county. Five parishes (the second smallest administrative units) of Kashaari county, namely Rwenjeru, Nyabuhaama, Mabira, Kakyerere and Rwenshanku, were randomly selected for inclusion in the study.

Study design, sampling and sample size determination

The sample size was determined using Win Episcope 2.0 software. Parasitological prevalence of AAT in Mbarara district was previously reported at 5.6% (Waiswa & Katunguka-Rwakishaya 2004). As the detection rate of trypanosome infections using polymerase chain reaction (PCR) is about twice that of parasitological techniques (Clausen *et al.* 1998; Picozzi *et al.* 2002; Solano *et al.* 1999), anticipated prevalence of bovine trypanosomiasis of 11.2%, the precision of the sample estimate (one half-length of the 95% confidence interval) of 5 percentage points, total number of cattle in the district (N) of 74 996 (Ministry of Agriculture, Animal Industry and Fisheries [MAAIF] & Uganda Bureau of Statistics [UBOS] 2009) were used to estimate sample size. The minimum number of cattle in the sample (n) was determined to be 153. Three to seven heads of cattle (number selected proportional to herd size) were randomly selected from each farm across the five randomly selected parishes, giving a total of 55 participating farms and 295 heads of cattle.

Cattle blood sample collection

About 125 µL of blood were collected from the middle ear vein of 295 cattle using capillary tubes and applied onto the sample area of the classic Whatman FTA® cards (Whatman BioScience Ltd, Cambridge, UK) (Ahmed *et al.* 2011; Muhanguzi *et al.* 2014; Picozzi *et al.* 2002). Each sample was applied in the designated sample area, avoiding sample cross-contamination. The four blood samples collected on each of the FTA® cards were allowed to air-dry thoroughly at ambient temperatures and labelled serially from 1 to 295 by the name of the farm, the parish and the date of collection. Samples were packed in foil pouches with a silica gel desiccant (Sigma-Aldrich, St Louis, USA) and shipped to the University of Edinburgh, United Kingdom for analysis.

DNA extraction

Deoxyribonucleic acid (DNA) was extracted and eluted from FTA® sample discs according to a protocol previously described by Becker *et al.* (2004). Briefly, each FTA® card was placed on a supporting base (Whatman BioScience Ltd) and from each of the individual samples five discs were punched out using a Harris 3.0-mm Micro Punch (Whatman BioScience Ltd) and discharged into 1.5-mL Eppendorf tubes. The Micro Punch was cleaned after punching each sample by punching at least the same number of discs from a clean filter paper (also see Muhanguzi *et al.* 2014). A negative control was prepared by punching the same number of discs from a sterile chromatography paper (Whatman BioScience Ltd) and was processed together with field samples. Samples were incubated twice for 15 min with 1.0 mL FTA® Purification Reagent (Whatman BioScience Ltd) at room temperature, followed by two rinses of 15 min with 1.0 mL TE-1 buffer (10 mM Tris–HCl, 0.1 mM EDTA, pH 8.0) at room temperature. After drying for 45 min at 37 °C or at room temperature overnight, the test sample or control discs were boiled at 90 °C for 30 min in DNA Engine Dyad® Cycler PTC-0221 (Bio-Rad Laboratories Inc., Hercules, USA) in 100 µL of 5% (w/v) aqueous suspension of Chelex 100 resin (Sodium form, 50–100 Dry mesh; Bio-Rad Laboratories Inc.). Eluted DNA samples were kept at -20 °C for PCR analyses or 4 °C if they were to be analysed within a few days after extraction (Ahmed *et al.* 2011; Becker *et al.* 2004; Muhanguzi *et al.* 2014; Picozzi, Carrington & Welburn 2008).

Internal transcribed spacer - polymerase chain reaction for trypanosome detection

Eluted DNA samples were screened for different trypanosome species using a single pair of primers (ITS1 CF, 5´CCGGAAGTTCACCGATATTG-3´ and ITS1 BR, 5´TTGCTGCGTTCTTCAACGAA-3´) previously designed to amplify internal transcribed spacer (ITS1) of different trypanosomes ribosomal deoxyribonucleic acid (rDNA) (Njiru *et al.* 2005). The 250, 480 and approximately 700 base pair fragments corresponding to *T. vivax*, *T. brucei s.l.* and *T. congolense* (*savannah*) were generated by ITS1 CF/BR primers, priming the ITS1 of rDNA of different trypanosomes in all infected samples. The PCR was carried out in 25 µL reaction volume, 20 µL of which was the PCR master mix containing 10x-reaction buffer (670 mM Tris-HCl pH 8.8, 166 µM $[NH4]_2SO_4$, 4.5% Triton X-100, 2 mg/mL gelatine) (Fisher Biotech), 1.0 mM $MgCl_2$, 200 µM of each dNTP, 5 µM each of the CF and BR primers, 0.5 U of *Taq* DNA polymerase (Fisher Biotech), 15.2 µL RNase-free water and 5 µL of extracted test sample DNA or positive control DNA or negative control eluate. PCR was carried out in a DNA Engine Dyad® Cycler (PTC-0221, Bio-Rad Laboratories Inc.) at cycling conditions including a denaturation step at 95 °C for 5 min, 35 cycles of denaturation at 94 °C for 40 s each cycle, annealing at 58 °C for 40 s, extension at 72 °C for 1.5 min and a final elongation step at 72 °C for 5 min (Njiru *et al.* 2005). PCR products were electrophoresed in 1.5% agarose (Bio Tolls Inc., Japan), stained in GelRed™ (Biotium Inc., Hayward, USA) and visualised on an ultraviolet transilluminator for fragment size determination (also see Muhanguzi *et al.* 2014).

Questionnaire data acquisition

A structured questionnaire was administered to 55 household heads to capture data on cattle management indicators, including household identification, number of cattle owned, cattle breeds, cattle age groups as well as acaricide, insecticide and trypanocidal drug usage.

Data analysis

Microsoft Excel™ spreadsheets were used for raw data entry and management. Questionnaire data was then exported to Statistical Package for Social Sciences (SPSS) version 16.0 for univariate and bivariate analyses. The data was then summarised into proportions and the prevalence of each parasite species was analysed in relation to independent variables such as age of the animal, acaricide and trypanocidal usage by chi square (χ^2) test. Prevalence data by ITS1-PCR were summarised into percentages and confidence intervals using Epidemiological Package for Information (EpiInfo™) version 3.5.

Ethical clearance

This study was approved by the Uganda National Council for Science and Technology (UNCST) on 15 December 2011 (HS 1098).

Results

Predictors of cattle trypanosome infections in Kashaari county, Mbarara district

The cattle herd size of the sampled households was highly variable, ranging from 3 to 260 (average: 50 cattle). About 87% of the farmers kept goats and the rest kept sheep as well. The average goat herd size was 20 whilst that of sheep was five. The majority of cattle kept were Ankole long-horned and Friesian hybrids (95.3%) whilst the rest were Ankole long-horned breed (4.7%). Farm lands were predominantly paddocked and most farmers (92%) practised restricted grazing. The rest of the farmers either grazed their livestock (cattle, sheep and goats) communally (5%) or tethered them (1.8%). There was no statistical association between risks of infection with any trypanosome species and different predictors of infection (breed, trypanocidal and acaricide usage and management system). The analysis of the predictors of trypanosome infections in 295 cattle sampled from 55 farms in Mbarara district, south-western Uganda is summarised in Table 1.

Overall trypanosome prevalence in Kashaari county

Of the 295 cattle screened, 6 (2.0%; 95% CI; 0.7% – 4.4%) were positive for *T. vivax* and 1 of these 6 was co-infected with *T. brucei s.l.* (0.3%; 95% CI; 0.0% - 1.9%). The overall prevalence of trypanosome species was 2.4% (95% CI; 1.0% – 4.8%).

Trypanosome prevalence by age and parish of origin

Trypanosome prevalence in different parishes was variable, ranging from 1.6% to 3.9%. There was no significant difference in the prevalence of different trypanosome species in different parishes from which cattle were sampled (χ^2 = 2.297; df = 4; p = 0.681). The variation in the prevalence of trypanosomes in different age groups was lower, ranging from 1.6% (calves < 0.5 years) to 3.4% (cattle > 2 years of age). The distribution of trypanosome infections by age and parish of origin is summarised in Table 2.

TABLE 1: Predictors of trypanosome infections (295 cattle sampled from 55 farms).

Species	Sub-predictor	Number		Positive†, (n)†	%	95% CI	χ^2	df	p-value
		n	%						
Breed	ALH	14	4.7	1.0	7.1	0.2–33.9	Ref	1.0	-
	Hybrids of ALH and HF	281	95.3	5.0	1.8	0.6–4.1	1.93	1	0.165
Trypanocidal drug usage	Do not use	27	49.1	4.0	14.3	4.0–32.7	Ref	1.0	-
	Use	28	50.9	2.0	7.4	0.9–24.3	0.669	1	0.413
Acaricide or insecticide usage	Amidines	6	10.9	2.0	33.3	4.3–77.7	Ref	1.0	-
	Pyrethroid	49	89.1	4.0	8.2	2.3–19.6	2.9	1	0.091
Management system	Communal grazing	1	1.8	0.0	0.0	-	Ref	1.0	-
	Paddocking	51	92.7	6	11.8	4.4–23.9	0.528	2	0.v768
	Tethering	3	5.5	0.0	0.0	-	na	-	na

†, Positive for either *Trypanosoma vivax* or *Trypanosoma brucei s.l.*
ALH, Ankole-long- horned; HF, Holstein Friesian; *df*, degrees of freedom; na, not applicable.

TABLE 2: Distribution of trypanosome infections by age and parish of origin.

Prevalence	Sub-group	Number sampled	Positive (n)	% Prevalence	95% CI
By parish of origin	Rwenjeru	51	2	3.9	0.5–13.5
	Nyabuhama	58	0	0.0	-
	Mabira	45	1	2.2	0.1–11.8
	Kakyerere	78	2	2.6	0.3–9.0
	Rwenshanku	63	1	1.6	0.0–8.5
By age	< 0.6 years	29	1	3.4	0.1–17.8
	0.6–1.0 years	41	1	2.4	0.1–12.9
	1.1–2.0 years	43	1	2.3	0.1–13.5
	> 2.0 years	182	3	1.6	0.3–4.7

Discussion

To establish the actual burden of AAT in south-western Uganda and the level of trypanocidal drug and acaricide usage, 295 cattle were sampled and examined by ITS1-PCR for different trypanosomes. Data gathered by questionnaires administered to 55 household heads of selected farms provided an understanding of the level of acaricide and trypanocidal drug usage, breeds of cattle kept, cattle management systems and cattle age demographics as the main predictors of infection with different trypanosome species.

The prevalence of bovine trypanosomes in Kashaari county, Mbarara was found to be 2.4%; *T. vivax* and *T. brucei s.l.* were the only trypanosome species detected. The prevalence reported here is lower than that previously reported (Waiswa & Katunguka-Rwakishaya 2004). This could be explained by the time-lag effect and shift from traditional husbandry with communal grazing to more restrictive grazing (paddocking of farm lands and tethering). Change from keeping local breeds to exotic breeds necessitated by the drive to increase milk production has resulted in farmers resorting to intensive use of acaricides or insecticides because exotic breeds are more susceptible to TTBDs (Muhanguzi, Ikwap, Picozzi & Waiswa 2010; Muhanguzi, Matovu & Waiswa 2010). This could further explain why the prevalence of bovine trypanosomes and their diversity reported in this study is lower and narrower respectively than reported previously. However, it was not possible to show this statistically because all the cattle kept in the study area are predominantly of one major breed (Ankole long-horned and Friesian hybrids) and are largely under restricted grazing with intensive use of acaricides and trypanocides. Prevalence of *T. vivax* was higher than the rest of the species probably because it has previously been reported to be mechanically transmitted, making it hard to control even in areas with intense vector control methods (Desquesnes & Dia 2003).

Slightly higher infections were detected in Rwenjeru than in any other parishes (Table 2). This parish is situated in Biharwe sub-county, very close to Lake Mburo National Park. Wildlife has been reported to be important in maintenance and spread of AAT (Anderson *et al.* 2011).

Hybrids of Ankole long-horned cattle and Holstein Friesians and farms that were using amidines for TBD control were associated with slightly higher risk of infection with different trypanosome species, although this association was not statistically significant. Long-horned cattle and other local cattle breeds have been reported to be more resistant to AAT than exotic breeds and their hybrids with local breeds (Magona *et al.* 2003; Magona, Walubengo & Odimim 2004). This is in agreement with our current finding. However, it was not possible to show this statistically because all the cattle kept in the study area were predominantly of one major breed (hybrids of Ankole long-horned cattle and Holstein Friesian). Amidines are not active against tsetse flies as pyrethroid and cypermethrin formulations are (alfacypermethrin, deltamethrin and flumethrin) (Torr *et al.* 2002). This probably explains why farms that only used amidines for tick and TBD control had more trypanosome infections.

Conclusions

The rather low prevalence of trypanosome infections in cattle in Mbarara district, south-western Uganda is likely to be as a result of cattle production intensification with paddocking of farm lands and extensive use of trypanocides and pyrethroid insecticides for simultaneous TTBD control. This systematic change in management system has been necessitated by the change-over of breeds from Ankole long-horned cattle to Holstein Friesians and their hybrids with the former, which are less resistant to TTBDs than local breeds (Ankole long-horned cattle). It was not possible to show this statistically because all the cattle kept in the study area were predominantly of one major breed (hybrids of Ankole long-horned cattle and Holstein Friesians) and were largely under restricted grazing with intensive use of acaricides and trypanocidal drugs. A broader study to explore AAT epidemiology in the larger part of the south-western dairy farming communities is recommended.

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Competing interests

The authors hereby declare no competing interests. The sponsors had no role in the study design, data collection and analysis, decision to publish or preparation of the manuscript.

Authors' contributions

R.A.A. (National Livestock Resources Research Institute), D.M. (Makerere University), E.M. (University of Edinburgh), C.W. (Makerere University) and J.F. (University of Edinburgh) conceived of and designed this study. R.A.A. carried out blood sample collection and E.M. and J.F. carried out PCR analysis. D.M., R.A.A. and E.M. carried out statistical analysis. All the authors participated in the manuscript write-up and final approval.

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