Molecular identification of bovine trypanosomes in relation to cattle sources in southwest Nigeria

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ABSTRACT

Bovine trypanosomosis is a problem in the livestock industry in Nigeria. A longitudinal survey of cattle sampled during the wet and dry seasons was conducted from April 2016 to March 2017. Blood samples were collected by random sampling from 745 cattle in southwest Nigeria and screened for trypanosomes by internal transcribed spacer–polymerase chain reaction (ITS-PCR). Cattle positive for *Trypanozoon* DNA were further screened with the Rode Trypanozoon antigen type (RoTat) 1.2 PCR and *Trypanosoma brucei gambiense* glycoprotein (TgsGP) genes for *T. evansi* and *T. b. gambiense* respectively. Trypanosome DNA was amplified in 23.8% (95%CI: 20.8–26.9) of cattle with significantly higher prevalence in wet season (95%CI: 22.9–30.8) when compared to the dry season (95%CI: 14.3–23.6). A high prevalence was observed in Fulani cattle farms 54.1% (95%CI: 42.78–64.93%) while the prevalence was lower in institutional farms 14.7% (95%CI: 10.10–20.97%). *Trypanosoma vivax* was the most prevalent trypanosome observed (11.54% (95%CI: 9.44–14.04%)), followed by *T. congolense* 8.5% (95%CI: 6.67–10.67%) *T. b. brucei* 4.8% (95%CI: 3.51–6.62%) and *T. evansi* 1.74% (95%CI: 1.02–2.96%). Mixed infections were observed in 2.8% (95%CI: 1.85–4.27%) of cattle. Seasonal variation revealed a predominance of *T. congolense* and *T. vivax* in wet and dry season, respectively. The high prevalence of *Trypanosoma* species in cattle indicates a need for expanded surveillance for AAT in southwest Nigeria. Migration, settlement patterns, increased marketing and management types were some of the risk factors identified for AAT.

1. Introduction

African animal trypanosomosis (AAT) is a major constraint to agricultural development and a threat to food security in sub-Saharan Africa. Losses to livestock producers and consumers have been estimated to exceed US$ 1.3 billion per year [1,2]. Production losses due to trypanosome infections in cattle have been estimated to be approximately 20% across a range of parameters, including draft power, calving rate, milk production, morbidity and mortality [3]. Approximately one-third of the land mass in sub-Saharan Africa is infested with tsetse flies [4]. This makes the growth and sustainability of livestock industry difficult in sub-Saharan Africa.

Although the humid zone of Nigeria is considered unsuitable for rearing livestock, recent studies have revealed an increasing level of livestock production and mixed farming [5]. Fulani have adopted settlement plans in southern Nigeria especially in the rural areas with an advantage of marketing their animals without middlemen in urban centres, while also engaging in agro-pastoralism [5,6]. The presence of tsetse flies and other economically important biting flies increases trypanosomosis risk, consequently livestock owners have struggled with AAT over the years [7].

In the 1960s and '70s prevalence of 71.4% was reported in trekked cattle in northern Nigeria, and 64% in southwest Nigeria [8,9]. Studies revealed that after national control programmes were initiated in 1955 with subsequent follow ups in 1978 and 1987, there was a general reduction in trypanosomosis prevalence both in the northern and southern states [10,11]. Recent reports in Jos Plateau in northern Nigeria [12] and Ogun state, southwest Nigeria [5] suggest the disease is endemic with prevalence values of 46.8% and 31.6% respectively. However, most reports in southwest Nigeria were on abattoir cattle, in

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which animals were transported from the north and only stay for short period before being slaughtered [13,14].

In Nigeria, there is no comprehensive map of AAT distribution, even though there are outlines of tsetse distribution, these have not been updated since the 1970s [7,15]. The relative abundance of each trypanosome species can greatly be affected within and between geographical regions due to management type (zero-grazing, pastoralism, nomadism etc.), efficacy of trypanocides used in an area, frequency of insecticide application, seasonal variation and sensitivity of various diagnostic techniques [16].

Information is sparse on extensive molecular studies attempting to capture the prevalence of AAT and its species distribution in southwest Nigeria [17]. This study focused on four cattle groups with different management conditions; farm cattle, abattoir cattle, institutional cattle and market cattle (trade cattle). We used molecular PCR-based diagnostic approaches to detect, identify and report the prevalence of trypanosomes in cattle blood from southwestern Nigeria.

2. Materials and methods

2.1. Study site and sampling

The study area is southwest Nigeria comprising Oyo, Ogun, Osun, Lagos, Ondo and Ekiti states between latitude 6°63′94.66″N–7°67′77.14″N and longitude 3°14′23.18″E–5°20′74.13″E. The land area is approximately 78,000 km^2 with varying altitude of 450–1200 ft above sea level. The area where cattle samples were collected can be found between the Atlantic Ocean to the south, Republic of Benin to the west, Kwara state and river Niger to the north and Edo state to the east (Fig. 1). The cattle population in the study area was approximately 2.4 million [18], however, there are currently no accurate counts of the ownership of these cattle. The annual rainfall is between 1500 and 2000 mm and mean temperature between 26 and 30 °C. The rainy season is between April and October annually while dry season starts in November and ends in March.

Due to the lack of accurate information on the number and location of cattle, we targeted areas where cattle were known to be present across the six southwest states. Fifty cattle sites were identified; however, access could not be gained to all of them and we managed to sample from 36 cattle sites in total. The sites comprised of 16 cattle farms, 12 abattoirs, six cattle markets and two institutional farms. Blood samples were then collected at six points in Igangan, four points each in Eruwa and Adegbile, three points each in Igboora, Adebayo, Idiroko, Ponpoola, two points each in Ikere and one point each in another eight locations (Akinyele, Onyearugbulem, Sango, UI, FUNAAB, Sabo, Lafenwa and Agege) (Fig. 1). All cattle that took part in the study had either identification tags or were branded.

The abattoirs are located in urban areas where retailers and consumers come to buy meat. They have expanded areas for lairage where cattle are inspected prior to slaughter. The cattle markets are often positioned in the countryside because they require a large expanse of land, where different owners keep and at times nurture their animals before sale. There is continuous movement of people trading in the cattle markets. The cattle farms are often based in rural (characterised by large expanse of land with Fulani discrete settlement) and peri-urban settlements with dense vegetation where Fulani pastoralists move their cattle across rural-urban areas in search of feed and water and sometimes close to market areas to dispose of stock when necessary. Institutional cattle farms are based in universities with animals often being raised for research purposes and demonstration.

Longitudinal sampling was done in wet (April – October) and dry (November – March) seasons and an assumed mean trypanosomosis prevalence of 14% based on literature reports of field work specifically focused on states in southwest Nigeria between 1987 and 2016. Precision was set at 2.5% and at 95% confidence the number of animals to be sampled was 741.

2.2. Cattle blood sample collection

Blood was collected by venipuncture of the middle ear vein in which sterile capillary tubes were used to collect 2–3 drops of whole blood (125 μl) and placed on Whatman Chromatography FTA™ (Flinders Technology Associate) card (Sigma-Aldrich, USA). The cards were air-dried and then stored in sealed waterproof pouches containing silica gel desiccant (Sigma-Aldrich, USA) at room temperature until use as previously described [19].

In total 1200 (589 male and 611 female) cattle blood samples were collected, however, 745 samples (362 male and 383 female) underwent molecular examination. The following breeds were sampled: White Fulani, Sokoto Gudali, Red Bororo, Kuri, N'dama, Muturu and Ambala. Age was determined by dentition and categorised as follows; ≤ 1 year, > 1 and ≤ 3 years and > 3 years. Cattle were sampled during wet season (480 samples) and dry season (265 samples). Body condition were scored based on three main conditions (fat, medium and lean) as previously described in zebu breeds [20].

2.3. DNA extraction from FTA cards using chelex®100

DNA was extracted in four stages following the protocol of Ahmed et al. [19]. Briefly five 3 mm discs were punched from each blood spot and washed twice in FTA wash buffer for 15 min, the FTA wash was removed by washing twice for 15 min in 1 X TE (1.0 M Tris–HCl, pH 8), containing 0.1 M ethylene-diamine tetra-acetic acid (Tris-EDTA) buffer (Sigma-Aldrich Ltd. Gillingham, UK). DNA was eluted by adding 100 μl 5% Chelex into each PCR tube and heated to 90 °C for 30 min in a Peltier thermal cycler (MJ Research Inc., USA) [21]. The samples were then stored at −20 °C.

2.4. Amplification of DNA using PCR

2.4.1. ITS rDNA PCR analysis

Genomic DNA (5 μl) and 20 μl master-mix comprised the 25 μl final reaction volume. Master-mix contained 5 μl of 5 × Mango Taq buffer, 1 μl of 50 mM MgCl$_2$, 1 μl of 0.4 μM both forward 5′-CCG-GAA-GTT-CAC-CGA-TAT-TG-3′ and reverse 5′-TTG-CTG-CGT-TCT-TCA-ACG-AA-3′ primers [22], 0.2 μl of 25 mM dNTPs, 0.2 μl of 5 U/μl Taq DNA polymerase and 12.6 μl double distilled water. A Bio-Rad Dyad Peltier Thermal cycler (MJ Research Inc., USA) was used for the PCR cycling. Thermal reactions involved an initial denaturation step of 72 °C for 5 min followed by 35 cycles of 94 °C for 40 s, 58 °C for 40 s, 72 °C for 90 s and a final extension step of 72 °C for 5 min.

2.4.2. TgsGP DNA PCR analysis

Trypanozoon positive samples were further tested using TgsGP PCR with an expected band size of 308 bp. This PCR allows identification of Type I *T. b. gambiense* [23]. PCR master mix in a 25 μl final volume contained 2.5 μl of 10 × CoralLoad PCR buffer (Qiagen), 0.3 μl of 5 U/μl Hotstar® DNA polymerase, 0.75 μl of 50 mM MgCl$_2$ (Bioline, UK), 0.2 μl of 25 mM deoxynucleoside triphosphates (dNTPs) (Rovalab, Germany), 5 μl of 10 pmol/μl of forward 5′-GCT-GCT-GTG-TTC-GGA-GAG-C-3′ and reverse 5′-GCC-ATC-GTG-CTT-GCC-GCT-C-3′ primers [23], 17.75 μl of double distilled water (Sigma-Aldrich, UK) and 1 μl genomic DNA. The PCR reactions involved 95 °C denaturation step for 15 min, annealing step of 45 cycles of 94 °C for 60 s, 63 °C of 60 s, 72 °C of 60 s and final extension step of 72 °C for 10 min.

2.4.3. RoTat 1.2 gene PCR analysis

All *Trypanozoon* positive samples on ITS1 were screened with RoTat 1.2 PCR with an expected band size of 205 bp. Master mix in a 25 μl final volume contained 5 μl of 5 × Mango Taq buffer, 0.2 μl of 25 mM dNTPs, 0.2 μl of 5 U/μl Taq DNA polymerase, 11.6 μl distilled water, 2 μl of 0.8 μM both forward 5′-GCG-GGG-TGT-TTA-AAG-CAA-TA-3′ and reverse 5′-ATT-AGT-GCT-GCG-TGT-GTT-CG-3′ primers [24]. Cycling
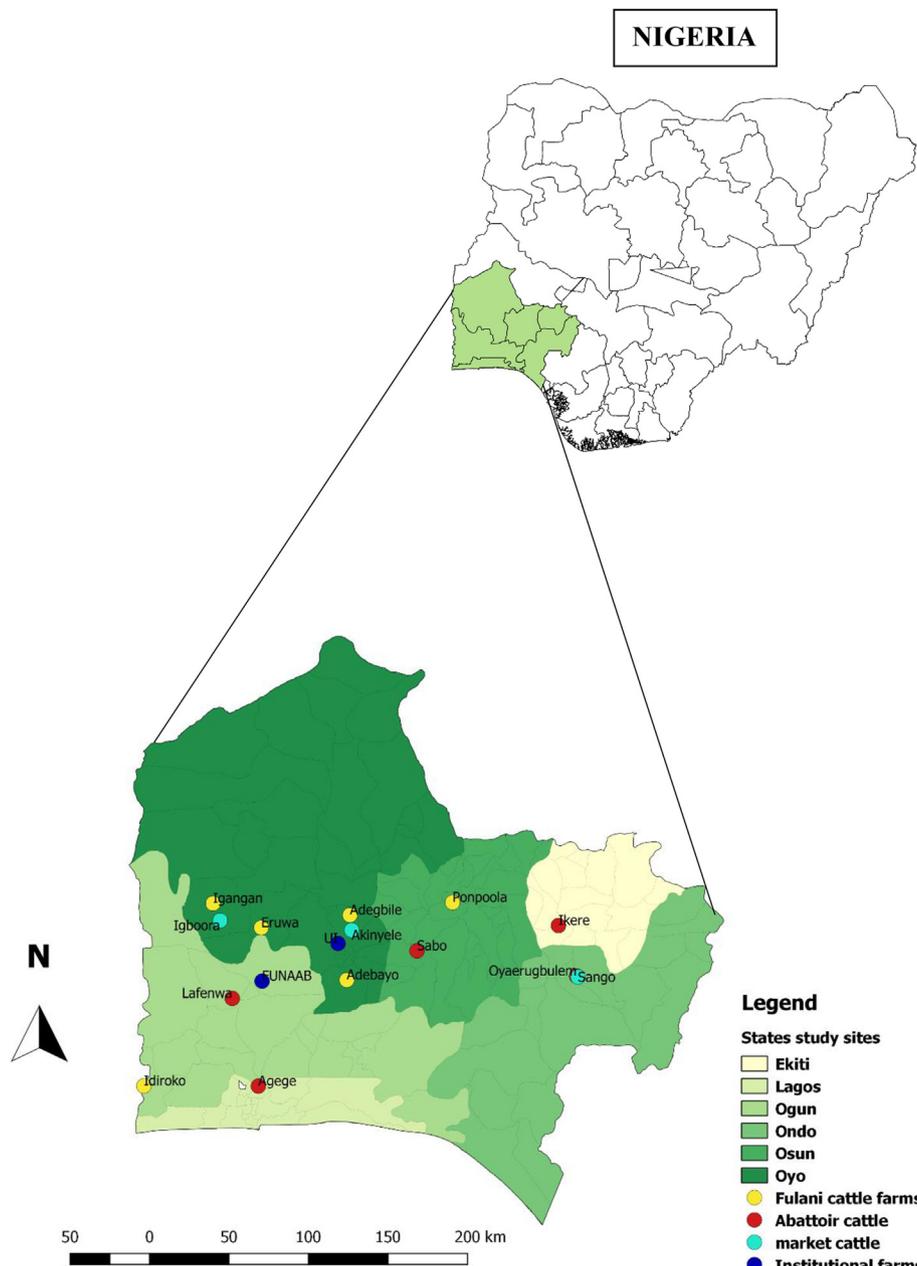
Fig. 1. Study locations of blood collection. The locations consist of six (6) Fulani cattle farms, two Institutional farms (FUNAAB and UI), five (5) abattoir cattle and three (3) market cattle.

conditions were as follows; 4 min at 94 °C for initial denaturation followed by 40 cycles of 1 min at 94 °C denaturation, 1 min at 59 °C primer-template annealing and 1 min at 72 °C polymerisation. Final elongation was done in 5 min at 72 °C.

2.5. Gel electrophoresis

PCR products were separated on a 1.5% agarose gel in 1 × Tris-borate–EDTA with GelRed™. Following electrophoresis, the bands were then read under a UV trans-illuminator (Gel-Doc™ 2000) with Quantity One software (Bio-Rad Laboratories, Inc.).

2.6. DNA purification with QIAquick® gel extraction kit and quantification

The gel products for positive *Trypanosoma* species were cut from the agarose gel into sterile 1.5 ml eppendorf tube. DNA was purified as described in the manufacturer's instructions and was quantified with

nanodrop spectrophotometer, ND-1000 (Labtech, United Kingdom). Only DNA products concentration of 10–20 ng/μl were outsourced for direct DNA sequencing.

2.7. Sequencing

The products of the ITS-PCR reactions were sent for Sanger sequencing (GATC, Germany)The sequences were viewed on CLC sequence viewer version 7.8.1 (Qiagen, Germany). Blast searches were run using megablast of GenBank database to search for highly similar sequences.

2.8. Statistical analysis

Log-transformed regression analysis was used for analysing significant differences in the prevalence of trypanosomosis for cattle sources, sampling states, age group, breed and body score, while
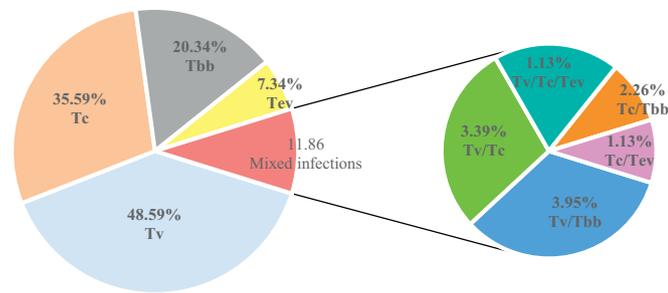
Fig. 2. Distribution of *Trypanosoma* species among the 177 positive cattle in southwest Nigeria. Abbreviations: Tc, *T. congolense*; Tv, *T. vivax*, Tbb, *T. brucei brucei*; Tev, *Trypanosoma evansi* and +, combined infection.

Tukey's multiple comparison ANOVA test was used for pairwise analysis. Pearson chi-square and odd-ratios of differences in gender and seasonal variations were reported. Species distribution differences among cattle sources and season were analysed with Pearson chi-square. Graphpad prism version 5 (San Diego, USA) and WINPEPI statistic package (United Kingdom) was used for all analyses. Microsoft Excel spread sheet was used to manage raw data and Wilson confidence interval was used to determine 95% confidence interval for descriptive analysis. A *P*-value < .05 was considered to be statistically significant.

3. Results

3.1. Trypanosome prevalence

The PCR analysis of 745 bovine blood samples in southwest Nigeria revealed that 177 cattle were positive for trypanosome DNA giving an overall prevalence of 23.8% (95%CI: 20.8–26.9). The overall trypanosome species distribution found within the 177 positive cattle is shown in Fig. 2. No *Trypanozoon* positive cattle were found to be positive for TgsGP PCR. *T. vivax* showed the highest prevalence of 11.5% (95%CI: 9.4–14.0%), followed by *T. congolense* (8.5%, 95%CI: 6.7–10.7%), *T. b. brucei* (4.8%, 95%CI: 3.5–6.6%) and *T. evansi* (1.74%, 95%CI: 1.02–2.96%). There were several types of mixed infections observed within the sampled cattle, these included infections of *T. vivax* and *T. b. brucei* (0.94%, 95%CI: 0.5–1.9), *T. vivax* and *T. congolense* (0.7%, 95%CI: 0.3–1.6), *T. congolense* and *T. b. brucei* (0.5%, 95%CI: 0.2–1.4), *T. congolense* and *T. evansi* (0.3%, 95%CI: 0.1–1.0). There was also triple infection consisting of *T. vivax*, *T. congolense* and *T. evansi* (0.3%, 95%CI: 0.1–1.0) (Table 1). There was no significant difference between females (25.6%, 95%CI: 21.5–30.2) having an insignificantly higher prevalence compared to males (21.8%, 95%CI: 17.9–26.4) (Table 2).

3.2. Seasonal prevalence of bovine trypanosomosis

Cattle were more likely to be positive ($X^2 = 6.301$, $P = .012$) in the wet season (26.7%, 95%CI: 22.9–30.8) than the dry season (18.5%, 95%CI: 14.3–23.6)(Fig. 3). However, there were variations dependent on the species of trypanosomes involved. *T. vivax* was observed to predominate in dry season with a prevalence of 14.7% (95%CI: 10.96–19.49) and significantly different ($X^2 = 4.056$, $P = .044$) from 9.8% (95%CI: 7.4–12.8) observed in the wet season. *Trypanosoma congolense* had a higher prevalence in the wet season with 11.0% (95%CI: 8.5–14.2) of cattle infected when compared ($X^2 = 11.651$, $P = .001$) to the dry season where 3.8% (95%CI: 2.1–6.8) were infected. *T.b. brucei* had a prevalence of 6.9% (95%CI: 4.9–9.5) in the wet season which wassignificantly higher ($X^2 = 12.245$, $P < .0001$) than 1.1% (95%CI: 0.4–3.3) in the dry season. *T. evansi* (1.74%, 95%CI: 1.0–3.0) was only found in the wet season (Fig. 3).

Table 1
Trypanosome species, source, state and breed prevalence of bovine trypanosomosis in southwest Nigeria.

Factor	Indices	Number sampled	Number positive	Prevalence	P-value
Species	*T. vivax*	745	86	11.5*	$X^2 = 291.00$,
	T. congolense	745	63	8.5*	$df = 8$,
	T. b. brucei	745	36	4.8*	$P < .0001$
	T. evansi	745	13	1.7	
	T. vivax/T. b. brucei	745	7	0.9	
	T. vivax/T. congolense	745	6	0.8	
	T. congolense/ T. b. brucei	745	4	0.5	
	T. congolense/ T. evansi	745	2	0.3	
	T. vivax/T. congolense/T. evansi	745	2	0.3	
Sources	Abattoirs	306	83	27.1*	$P = .350$;
	Fulani cattle farms	74	40	54.1*	S.E = 0.257;
	Institutional farms	163	24	14.7	95%CI: −1.061–0.440
	Cattle markets	202	30	14.9	
States	Oyo	210	56	26.7	$P = .495$;
	Ogun	182	45	24.7	S.E = 0.081; CI:
	Lagos	251	55	21.9	−0.234–0.112
	Ondo	60	9	15.0	
	Osun	29	10	34.5	
	Ekiti	13	2	15.4	
Breeds	White Fulani	480	119	24.8	$P = .525$;
	Sokoto Gudali	111	24	21.6	S.E = 0.039;
	Red Bororo	121	30	24.8	95%CI:
	Ambala	8	0	0.0[a]	−0.145–0.085
	Kuri	14	3	21.4	
	Muturu	10	0	0.0[a]	
	N'dama	1	1[a]	–	

Symbols: (*) indicates pairwise significance ($P < .05$) with Tukey multiple comparison test, ([a]) indicates data not included in analysis because of small numbers observed in sampled population.

Table 2
Cattle indices in relation to bovine trypanosomosis in southwest Nigeria.

Factor	Indices	Number sampled	Number positive	Prevalence	P-value
Body scores	F (fat)	124	12	9.7*	$P = .383$;
	M (medium)	257	72	28.0	S.E = 0.333;
	L (lean)	364	93	25.5	95%CI: −1.620–2.591
Age groups	≤ 1 year	69	13	18.8*	$P = .272$;
	≤ 3 years	289	69	23.9	S.E = 0.060;
	> 3 years	387	95	24.5	95%CI: −0.249–0.513
Seasons	Wet	480	128	26.7*	$P = .012$,
	Dry	265	49	18.5	$X^2 = 6.30$, OR = 1.60 (95%CI: 1.09–2.37)
Sex	Male	362	79	21.8	$P = .228$,
	Female	383	98	25.6	$X^2 = 1.45$, OR = 0.81 (95%CI: 0.57–1.16)

Symbols: (*) indicates pairwise significance ($P < .05$) with Tukey multiple comparison test.

3.3. Trypanosome prevalence based on cattle sources

Even though the log-transformed regression analysis overall prevalence shows no significant difference in cattle sources, pairwise
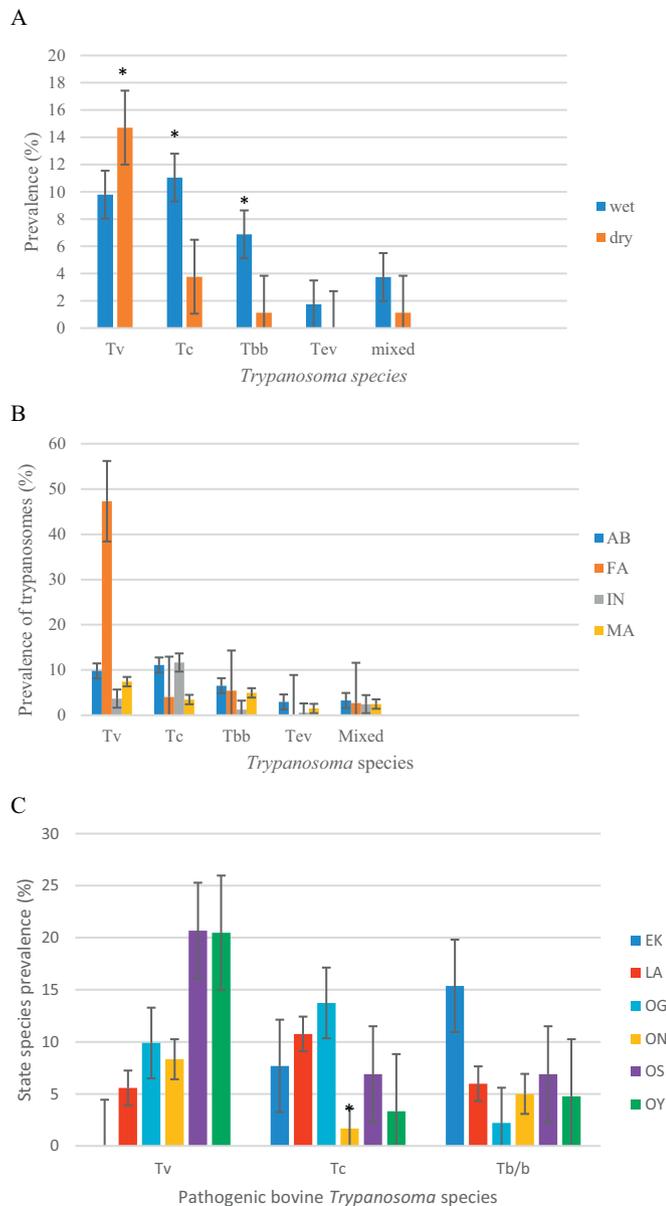
Fig. 3. A Seasonal distribution of *Trypanosoma* species prevalence in southwest Nigeria. B. *Trypanosoma* species distribution in cattle from southwest Nigeria in relation to source and managemental practices. 3C. Southwest states pathogenic *Trypanosoma* species distribution. *indicates significant difference (P < .05). Abbreviations: Tc, *T. congolense*; Tv, *T. vivax*, Tbb, *T. brucei brucei* and Tev, *Trypanosoma evansi*. AB – abattoirs, FA – Fulani cattle farms, IN – institutional cattle farms and MA – cattle markets. EK- Ekiti, LA- Lagos, OG-Ogun, ON- Ondo, OS- Osun and OY- Oyo.

Tukey multiple comparison test showed differences among some groups (Table 1). The prevalence of trypanosomes in abattoir cattle was significantly higher than those of institutional cattle farms (P = .002) and market cattle (P = .001) but significantly lower compared with Fulani cattle farms (P < .0001). It was observed that the infection rate in Fulani cattle (54.1%, 95%CI: 42.78–64.93%) was significantly higher (P < .0001) than other cattle sources, namely institutional cattle farms 14.7% (95%CI: 10.10–20.97%) andcattle markets 14.9% (95%CI: 10.61–20.41%). There was no significant difference (P = .973) in the prevalence between institutional farms and cattle markets (Table 1).

In relation to trypanosome species distribution from cattle sources (Fig.3B), prevalence of *T. vivax* was highest in Fulani cattle farms with 47.3% (95%CI: 36.3–58.5) while *T. congolense* was predominant in

institutional cattle farms and abattoir cattle where the prevalence was found to be 11.7% (95%CI: 7.6–17.5) and 11.1% (95%CI: 8.1–15.1) respectively. Prevalence was lower in Fulani cattle and cattle from the market. Prevalence of *T. b. brucei* infections were similar in all four cattle sources. *Trypanosoma evansi* was found in 2.9% (95%CI: 1.6–5.5), 1.5% (95%CI: 0.51–4.3) and 0.6% (95%CI: 0.1–3.4) of abattoir, market and institutional cattle respectively. No Fulani cattle were infected with *T. evansi*.

3.4. State prevalence of bovine trypanosomosis

State prevalence shows a sequence of Osun > Oyo > Ogun > Lagos > Ekiti > Ondo. However, no significant difference was observed (Table 1). Fig.3C shows the prevalence by state of the common trypanosomes in cattle population under study. Briefly the prevalence of *T. vivax* was highest in Osun (20.7%, 95%CI: 9.9–38.4) and Oyo (20.5%, 95%CI: 15.6–26.4) states and was due to the presence of Fulani cattle which showed high prevalence. The prevalence of *T. congolense* was highest in Ogun state at 13.7% (95% CI: 9.5–19.5) followed by Lagos 10.7% (95%CI: 7.5–15.2). *T. b. brucei* was highest in Ekiti (15.4%, 95%CI: 4.3–42.2) followed by Osun state (6.9%, 95%CI: 1.9–22.0).

3.5. Cattle breeds distribution of bovine trypanosomosis

Trypanosome distribution among cattle breeds did not show a significant difference among the local trypanosusceptible breeds (White Fulani, Sokoto Gudali, Red Bororo) otherwise known as zebu types (Table 1). The taurine groups which are trypanotolerant (Kuri, Muturu and N'dama) could not be statistically analysed because of their small numbers in the livestock population. However, the overall prevalence for zebu group was 24.0% (95%CI: 21.05–27.28) which was not significantly higher ($X^2 = 0.860$, P = .354) compared to taurine groups 16.0% (95%CI: 6.40–34.65).

3.6. Body condition relationship to trypanosomosis

Clinical signs such as malnutrition, anaemia, dehydration and morbidity were prominent indices in cattle with lean body score. Cases of AAT without obvious clinical signs were observed in the medium body score. The PCR detection method revealed 9.7% (95%CI: 5.62–16.16%) cattle in fat body score to be positive of AAT. However, there was a significant increase in the prevalence when compared with those with medium body score ($X^2 = 16.366$, 28.2% (95%CI: 22.9–33.8), P < .0001) and lean body score ($X^2 = 12.699$, 25.6% (95%CI: 21.3–30.3), P < .0001) (Table 2).

3.7. Age differences in relation to bovine trypanosomosis

The proportion of cattle harbouring trypanosome infections of multiple species in this study were observed to vary with age group. Cattle ≤ 1 year had lower prevalence which is significantly comparable to others (Table 2). Those of > 1 and ≤ 3, and > 3 years do not vary significantly ($X^2 = 0.041$, P = .840).

3.8. Nucleotide sequence analysis

Fifteen *T. vivax* isolates were sequenced, of these eleven matched 99–100% with TvY486 (ILRAD 700) with accession number KM391828, these were observed in Oyo, Ogun, Osun and Ondo States. Matches to this isolate were found in all four cattle sources. Three *T. vivax* with 98% similarity, MBOV/ET/2012/AAU-CVMA/004 resembled isolate (KM391827) were observed in Lagos State from Agege abattoir which also had 98% similarity with another isolate in Ethiopia [25]. Meanwhile, one sequenced sample from Lafenwa abattoir, Ogun State had 100% similarity with the same description (similar query,

maximum score, total score, query cover and e-value) for five different isolates (KM391828, JX910375, JX910372, HE573019 and U22316), found in Ethiopia and Burkina Faso.

The *Trypanosoma congolense* positives in this study were not classified into either savannah or forest by ITS1-PCR. Analysis of the sequences generated from positives from Akinyele cattle market, Oyo State, UNAAB institutional farm, Ogun State showed ten out of the sixteen sequences to be 96–99% similarity with *T. congolense* riverine/forest-type from Kenya (U22319). Four sequences showed that *Trypanosoma congolense* isolates in FUNAAB institutional farm had 86% similarity and resembled a strain from Ghana (AB742531). Positive bands amplified from Fulani cattle at Eruwa and Igboora showed 81% similarity with an isolate (KX870079) from tabanids from South Africa and Zambia.

Twenty-six *Trypanozoon* isolates were sequenced, this consisted of thirteen that were positive for RoTat 1.2 and thirteen isolates were positive for ITS-PCR only. Thirteen of those positive for RoTat 1.2 showed high similarity (97–99%) with *T. evansi* isolated from a camel in Iran. These samples were collected from Agege abattoir ($n = 11$), Lagos State and Akinyele cattle market, Oyo State ($n = 2$).

Thirteen of those positive for ITS only showed high similarity with the *T. evansi* isolate from Iran (KX898420). Other matches observed at 98% include *T. evansi* isolate and *T. brucei*. These samples were collected from Agege abattoir, Lagos State ($n = 6$), Igboora farm ($n = 3$) and Akinyele cattle market, Oyo State ($n = 4$).

A total of seven sequences were submitted to the GenBank with the submission ID: SUB4467120. Accession numbers have been assigned to two *T. vivax* sequences (MH796907 and MH796908), four *T. congolense* sequences (MH79909-MH796912) and one *T. evansi* (MH796913).

4. Discussion

To the best of our knowledge, this is the first-time trypanosomes have been molecularly identified in southwest Nigeria in relation to cattle sources and seasonal variation. Ikede et al. [11] did examine cattle in southwest Nigeria using conventional microscopy observing a prevalence of 14.4%. The increased prevalence in this study (23.8%) is most likely due to the increased sensitivity of PCR over microscopic methods. In 2013 however, Takeet et al. [26] reported a prevalence of 76.6% in Abeokuta (a study area in southwest Nigeria) using species-specific primers. The higher prevalence could by due to several factors such as primers used, type of animal sampled and seasonal influence.

Cattle were more likely to be positive in the rainy season (although there were trypanosome species differences between seasons) and this may be due to an increased presence of vectors which could transmit trypanosomes. Previous work has shown that high temperatures and low humidity during the dry season restrict tsetse fly dispersal in the environment and also lower tsetse fecundity [27,28].

In our work *T. congolense* was observed to predominate in the wet season, while *T. vivax* had highest prevalence in the dry season. Seasonality could be the reason why authors have found differing results when investigating trypanosome species found during cattle surveys. Majekodunmi et al. [12] reported that the highest prevalence of *T. vivax* was in late wet season and that the highest prevalence of *T. congolense* was in the dry season from northern Nigeria. However, there were no significant differences between their three sampling points (dry season, early wet season and late wet season). A contributing factor to *T. vivax* abundance in dry season could be the presence of biting flies throughout the season that act as mechanical vectors. Anene et al. [29] suggested that bovine trypanosomosis is maintained in the herd during the dry season by tabanids and stomoxyine flies (while tsetse populations are suppressed).

The prevalence of the *Trypanozoon* group (6.6%, 95%CI: 5.0–8.6) reported in this study is higher than those reported in previous molecular studies in Nigeria [12,26]. None of the samples we tested were positive for TgsGP which would suggest that *T. gambiense* Type I is not present within the sampled cattle population. The study area has not reported any recent human sleeping sickness cases and there is no active screening within the human population for trypanosome infection. Studies have suggested that domestic livestock can be infected with *T. b. gambiense* [30] and that they can transmit *T. b. gambiense* to tsetse flies under laboratory conditions [31]. *T. evansi* has been previously molecularly identified in southeast Nigeria and southwest Nigeria and may be present across Nigeria [26,32]. When comparing molecular reports of bovine trypanosomosis in southwest Nigeria, the prevalence in Ogun state (24.7%) is lower compared to the report by Takeet et al. [26]. The highest prevalence was observed in Osun from this study (34.5%), while Ikede et al. [11] reported the highest prevalence in Ondo. Several factors (season, sampling and diagnostic technique, animal sampled etc.) could have impacted the dynamics of trypanosomosis in this study area.

Fulani cattle farms, where cattle are engaged in movements to different pastoral fields in search of grasses and water had the highest prevalence in the four systems examined. Researchers are often denied access to these animals by Fulani pastoralists in the southwest of the country, due to ethnocultural reasons. Hence few studies can be traced to sedentary farms owned by the Fulani [5,33]. Gathering of cattle at few watering points could also predispose cattle to trypanosomosis through increasing their contact with haematophagous flies [34]. Hence, livestock husbandry practices could have direct or indirect impact on regional prevalence of AAT at a given time. Activities such as transhumance, transportation of cattle from far north into regions with low prevalence due to marketing activities could have exacerbated infection. The lowest prevalence in institutional cattle farms could be due to monitoring of the animals with routine management which involves the use of trypanocides shortly before rain begins and early dry season. Bovine trypanosomosis has previously been observed to reduce as livestock stay longer in the markets [35], because the transmitting vector distribution is lower in the areas, as there is a dense human population, coupled with persistent trypanocide treatment of herds. Furthermore, most trade cattle are transported in lorries from northern Nigeria to southern markets thus avoiding the risk of infection in transit across the *Glossina morsitans* belt [11]. The occurrence in abattoir cattle could be due to transport stress factors, high infection rate from the source or host immune response to old or new challenge. Oluwafemi et al. [36] reported higher prevalence of trypanosomosis in cattle that were to be slaughtered compared to sedentary cattle. Trypanotolerant breeds were clinically healthy and apparently have lower trypanosome infection rates compared to trypanosusceptible breeds in which some cattle with good body score were positive fortrypanosomosis. Our study reported significantly lower infection rate in calves ≤1-year old as compared to adults and aged cattle which is similar to previous reports [26,37,38]. Bovine trypanosomosis has several forms, either as acute or chronic disease and age group difference is important in its epidemiology. Lower infection in calves could be because of maternal immunity conferred from the cow and higher infection in adults could be due to accumulated longer exposure to tsetse areas.The higher prevalence in older cattle may also be due to them being more attractive to tsetse as bloodmeal sources [39]. Calves also do not accompany their owners on transhumance until after they are six months old and this may also play a role in their lower trypanosome prevalence. The prevalence of bovine trypanosomosis in terms of sex in this report revealed a non-significant higher percentage among the females when compared with males. This is the same as reported in previous studies in Nigeria [10,40,41].

The sequence analyses of *T. vivax* isolates (MH796907 and MH796908) which showed close similarity to GenBank matches of KM391828 and KM391827, respectively of the ITS gene copies, indicates that the use of generic primer is essential for detection of trypanosome population surveys. The same variability observed in *T. congolense* and *T. evansi* isolates showed that trypanosome survey in cattle have verified the extent of diversity in these trypanosomes compared to previously recognised species in the West Africa region.

5. Conclusions

Trypanosoma species distribution was influenced by seasonal variation, with *T. congolense* and *T. vivax* predominating in the wet and dry seasons respectively. Livestock sources in respect to management practices affect the prevalence of bovine trypanosomosis in geographical areas with time. The highest prevalence was found in Fulani cattle farms and this may be due to their use of transhumance in search of grass and water. Further investigations are required to implement a cost-effective control measures against bovine trypanosomosis in southwest Nigeria.

Declaration of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Ethical approval

The study was conducted with the permission of the University of Ibadan Animal Ethics Committee (UI-ACUREC/App/12/2016/05) and in line with the guidelines of the committee.

Informed consent

Cattle owners were duly consulted before blood collection and in cases of restriction, cattle were not bled.

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