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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 contained2.5 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