Research Article

Clinico-haematological manifestation of single and mixed *Trypanosome species* infection of pigs in Federal Capital Territory (Abuja) and Nasarawa State, Nigeria.

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Abstract

African Animal Trypanosomosis (AAT) is a chronic debilitating disease affecting the productivity of livestock. In order to provide useful information on the clinical manifestation, disease and species prevalence and haematological alterations associated with trypanosome infection in pigs from Federal Capital Territory and Nasarawa states, blood was collected from 395 pigs. Diagnosis of *Trypanosoma species* were done clinically, using microscopy, serology (immune trypanoslysis test) and molecular detection. Observed clinical manifestations were generalized alopecia, hyperemia of the skin, emaciation, ataxia, pale mucous membrane, hurdling, piloerection. A total of twenty-six pigs were found to be positive for either single or mixed trypanosome infections. None of the twenty-six trypanosome positive pigs was positive for *T. brucei gambiense* using the TgsGP PCR and Immune Trypanolysis test. In comparison with single and double infections, quadruple infection caused a significant decrease ($P < 0.05$) in Packed Cell Volume, Red Blood Cell Count, Haemoglobin Concentration, Mean Corpuscular Volume, Mean Corpuscular Haemoglobin and Mean Corpuscular Haemoglobin Concentrations. Pigs are reservoirs of trypanosome species that are pathogenic to other animal species but not humans. Clinicians should also acquaint themselves with current trend of clinical signs to avoid speculative diagnosis which potentiates drug resistance.

Keywords: *Trypanosoma species*, Pigs, Abuja, Nasarawa

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Résume

La trypanosomose animale africaine (TAA) est une maladie chronique débilitante affectant la productivité du bétail. Afin de fournir des informations utiles sur les manifestations cliniques, la prévalence de la maladie et des espèces et les altérations hématologiques associées à l'infection trypanosomienne chez les porcs du Territoire de la capitale fédérale et des États de Nasarawa, du sang a été collecté sur 395 porcs. Le diagnostic des espèces de Trypanosoma a été effectué cliniquement, par microscopie, sérologie (test de trypanoslyse immunitaire) et détection moléculaire. Les manifestations cliniques observées étaient une alopécie généralisée, une hyperémie de la peau, une émaciation, une ataxie, une muqueuse pâle, une pellicule, une piloérection. Au total, vingt-six porcs se sont révélés positifs pour des infections trypanosomiennes simples ou mixtes. Aucun des vingt-six porcs positifs pour les trypanosomes n'était positif pour T. brucei gambiense en utilisant le test TgsGP PCR et l'immunotrypanolyse. En comparaison avec les infections simples et doubles, l'infection quadruple a entraîné une diminution significative (P <0,05) de l'hématocytes, du nombre de globules rouges, de la concentration en hémoglobine, du volume corpusculaire moyen, de l'hémoglobine corpusculaire moyenne et des concentrations moyennes d'hémoglobine corpusculaire. Les porcs sont des réservoirs d'espèces de trypanosomes pathogènes pour d'autres espèces animales mais pas pour les humains. Les cliniciens doivent également se familiariser avec la tendance actuelle des signes cliniques afin d'éviter un diagnostic spéculatif qui potentialise la résistance aux médicaments.

Mots-clés : espèces de Trypanosoma, porcs, Abuja, Nasarawa . Credit for Translation : Google Translate www.google.com

Introduction

African Trypanosomosis is a chronic debilitating disease of animals and humans (Jha et al., 2015; Mwai et al., 2015), which is caused mainly by *Trypanosoma vivax*, *Trypanosoma congolense* and *Trypanosoma brucei* (Nakayima et al., 2012; Kato et al., 2015) with a further three species (*T. simiae*, *T. godfreyi* and *T. suis*) commonly infecting pigs specifically. Clinical sign and pathology is infrequent and usually observed when pigs are infected with *T. simiae* (Hamil et al., 2013). These manifestations in pigs include anorexia, anaemia, fever, dullness, lethargy, depression, muscular weakness, central nervous manifestation, corneal opacity, abortion, enlargement of lymph nodes and acute death (Premaalatha et al., 2014).

Despite its role in food animal medicine, there are few reports on natural porcine trypanosomosis, when compared with reports in cattle, small ruminant and camels (Karshima et al., 2016). In pigs, reported prevalences are 16.6% reported in Karim Lamido of Taraba State (Karshima et al., 2016), 28.6% in the Koforidua and Adidome areas in the southern part of Ghana (Nakayima et al., 2012), and 4.3% reported in the western region of Ghana (Apaatah et al., 2020). Pigs have been reported to be reservoirs of the human-infective sub-species *T. b. gambiense* (Stephen, 1986; Karshima et al., 2016), in which pigs mostly remain asymptomatic and generally show low to very low parasitaemia when infected (Buscher et al., 2018). Pigs were first reported to be capable of acting as reservoirs of *Trypanosoma brucei gambiense* in 1986 (Stephen, 1966). This report was confirmed by Karshima et al. (2016) in pigs from Old Gboko (Benue State, Nigeria) – the location of its first report in humans in 1976 (Aiyedun and Amodu, 1976).

In addition to identification using molecular detection, this study identified the associated clinical signs and determined the haematological characteristics and prevalence of natural porcine trypanosomosis in Federal capital territory (Abuja) and Nasarawa state.

Materials and Methods

Ethical Approval

Ethical approval was from Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC) and given the approval number, ABUCAUC/2019/26

Study areas and population

The study was carried out in Federal Capital Territory (FCT) and Nasarawa State. FCT, located in the North-central part of the country (9°42 N, 7°292 E) while Nasarawa state lies between latitudes 7°50‘ and 9°50’N and longitude 6°54E. Pig population were cross breeds of Large White X Duroc, Duroc and Hampshire drawn from intensive farms within Gwagwalada, Kuje and Kwali Area Councils of Federal Capital Territory and Karu, Keffi and Nasarawa Local Government Areas of Nasarawa State.

Study design and sampling technique

A cross sectional study was carried out from November 2019 – June 2021. For this study, a systematic random sampling technique was used in the selection of study animals and 395 Pigs were sampled across this period in both FCT and Nasarawa State.

Clinical examination and Sample collection

Herd size, breed, history of use of trypanocidal drugs, production system and farm/herd locations were recorded. The animals were observed for abnormal signs which were then recorded. Blood samples were collected from the anterior vena cava of individual animals, out of which about 35ìl was dispensed unto Whatman Flinders Technology Associate (FTA) classic cards for molecular detection, and the remainder transferred into commercially prepared sample bottles containing Di-sodium salt of ethylene diamine tetra-acetate (EDTA, 1 mg/ml) for hematological and parasitological analysis

Parasitological diagnosis

The demonstration of the presence of *Trypanosoma* species, morphological identification and differentiation were done using Haematocrit Centrifugation Technique (Ledoka, 2009), buffy coat and Giemsa staining method (Woo, 1970). Stained slides were examined using oil immersion under x 100 microscopic magnification.

Haematology

This was carried out using the Veterinary Automated haematology analyzer (HI 2800, Pioway Medical Lab Equipment, China).

DNA extraction

DNA was extracted in four stages following the protocol of Ahmed et al. (2011). Following a series of washes, the supernatant was removed, and the tubes were centrifuged at 10,000 rpm for 15 sec before adding 130 ìL of a 10% Chelex® (Sigma)-TE suspension and 1.3 ìL of proteinase K (20 mg/mL, Bioline). The eluted DNA was transferred to a separate tube and stored at -20°C until further use.

Amplification of DNA with PCR

Internal Transcribed Spacer PCR

The PCR reactions that used 5x Firepol® mastermix were carried out at a final volume of 25 ìL each containing the following reagents: 2.5 ìL of 10X PCR Buffer (Bioline), 200 ìM of each of the deoxynucleoside triphosphates (dNTPs) (ThermoFisher), 1.2 mM of $MgCl_2$ (Bioline), 0.4 ìM of both the forward 52 -CCG-GAA-GTTCAC-CGA-TAT-TG-32 and reverse 52 -TTG-CTG-CGT-TCT-TCA-ACG-AA-32 primers (Njiru et al., 2004) and 10 ìL of BIOTAQ Red DNA Polymerase (Bioline). The PCR reactions were carried out on a BioRad T100TM thermal cycler, with an initial denaturation step at 95R”C for 5 min, 35 cycles of 95R”C for 30 sec, 55R”C for 30 sec and 72R”C for 30 sec followed by a final extension step of 72R”C for 3 minutes.

TgsGP PCR

Trypanozoon positive samples were subjected to *T. b. gambiense* reactions. In this, a final volume

of 25 ìL consisting of: 12.5 ìL of 5X firepol mastermix with 0.4 ìM of both the forward 52 -GCT-GCT-GTG-TTC-GGA-GAG-C-32 and reverse 52 -GCC-ATC-GTG-CTT-GCC-GCT-C-32 primers (Radwanska *et al.,* 2002) and 2 ìL of DNA template for the *T. b. gambiense* specific primers. The following cycling conditions were used: 95R"C for 3 minutes followed by 40 cycles of 95R"C for 30 s, 63R"C for 90 s, 72R"C for 70 s with a final extension step for 10 minutes. The amplified DNA from the PCR was resolved on 1% of UltraPureTM agarose gel (InvitrogenTM by Life TechnologiesTM, USA), and the images visualized in Gel DocTM XR (Bio-Rad Laboratories Inc., USA).

RoTat 1.2 gene PCR

All the Trypanozoon positive samples were subjected to screening using RoTat 1.2 PCR (band size of 205bp), which is specific for *T. evansi* strains. A final volume of 25 ìL contains 5 ìL of 5X Firepol® mastermix with 2ìl of 0.8ìM of the forward 52 -GCG-GGG-TGT-TTA-AAG-CAA-TA-32 and reverse 52 -ATT-AGT-GCT-GCG-TGT-GTT-CG-32 primers (Claes et al., 2004), 0.2ìl of 25mM dNTP, 0.2ìL of 5U DNA polymerase and 11.6ìl distilled water. The following cycling conditions were used: 94R"C for 4 minutes followed by 40 cycles of 94R"C for 60s, 59R"C for 60 s, 72R"C for 60 s with a final extension step for 5 minutes at 72R"C.

Agarose Gel Electrophoresis

PCR amplicons were separated on a 1.5% agarose gel stained with 10mg/ml ethidium bromide and using a 100 bp ladder to determine amplicon sizes. Following electrophoresis, the bands were then read under a UV trans-illuminator (Gel-Doc™ 2000) using a Chemi-Doc software (Bio-Rad Laboratories, Inc.).

Statistical analysis

Data was presented as mean ± SEM. One way ANOVA followed by Tukey's post-hoc test was used to analyze the data. GraphPad prism version 8.0.2 for windows (GraphPad software San Diego, California, USA) was used with $p < 0.05$ considered statistically significant.

Results

Prevalence of *Trypanosoma species* in Pigs using microscopy and PCR

Trypanosoma brucei was the only trypanosome identified on microscopy (Plate 1). The prevalence of Trypanosomes according to microscopy was 2.70% (5/185) in FCT and 3.33% (7/210) in Nasarawa state as compared to 9.19% (17/185) in FCT and 3.81% (8/210) in Nasarawa state based on PCR. In FCT, Porcine Trypanosomosis was attributed to single infections of *T. congolense* (1.08%). *T. simae* (0.54%), *T. evansi* (1.08%), *T. brucei* (5.41%) and double infection of *T. congolense* and *T. brucei* (1.08%) (Plate 2and 3; Table 1). However, in addition to single and double infection, there was a quadruple trypanosome infection involving *T. congolense*, *T. brucei*, *T. vivax* and *T. godfreyi* in Nasarawa state (Plate 3, Table 2). Using *T. brucei gambiense* specific primers for TgsGP-PCR, all Trypanozoon positive samples were negative to *T. brucei gambiense* (Plate 4)

Clinical signs associated with single, double and quadruple *Trypanosoma species* infection in Pigs

Out of the total number of 395 pigs sampled in FCT and Nasarawa state, 25 trypanosome-infected pigs showed signs of emaciation, huddling, piloerection, ataxia, pale mucous membrane, alopecia and hyperemia of skin (Plate 5 – Plate 7). Emaciation, pilo-erection and hyperemia of skin at prevalence of 78.6% (11/14), 71.4% (10/14) and 92.9% (13/14) respectively were observed to be associated with *T. brucei* single infection (Table 3). However, pale mucous membrane in addition to emaciation and pilo-erection at respective prevalence of 75% (3/4), 75% (3/4) and 75% (3/4) were observed in double infection of *T. congolense* and *T. brucei* (Table 4). The clinical signs observed in quadruple mixed trypanosome infection of pig were emaciation,

pale mucous membrane and rough hair coat (Table 5).

Mean haematology of single, double and quadruple *Trypanosoma species* infection in Pigs

The pig infected with mixed *T. congolense*/*T. brucei*/*T. vivax*/*T. godfreyi* had a lower PCV (18.23%±0.00) when compared to mean values of mixed *T. congolense*/*T. brucei* (25.23%±1.81), single *T. brucei* (38.21%±4.53), *T. evansi* (34.31%±0.86), *T. congolense* (31.38%±1.22), uninfected (43.01%±3.21) pigs and *T. simiae* (35.31%±0.00) infected pig. Similarly, quadruple trypanosome infection led to a lower RBC count when compared with mean values of other trypanosome species infected and uninfected pigs.

Amidst the infected group, there was also significant variation in the mean RBC count of pigs infected with mixed *T. congolense*/*T. brucei* ($3.31x10^6$±0.61) when compared with single *T. congolense* ($4.27x10^6$±0.12), *T. brucei* ($5.83x10^6$±0.56) and *T. evansi* ($5.55 x10^6$±0.46) infected pigs. This study observed a lower haemoglobin concentration (5.21mg/dl±0.00) with mixed *T. congolense*/*T. brucei*/*T. vivax*/*T. godfreyi* when compared with that of mixed *T. congolense*/*T. brucei* (8.72mg/dl±1.09), *T. congolense* (10.19mg/dl±0.32), *T. evansi* (14.10mg/dl±0.61), *T. brucei* (12.22mg/dl±2.10) infected and uninfected (14.22mg/dl±1.01) pigs (Table 6).

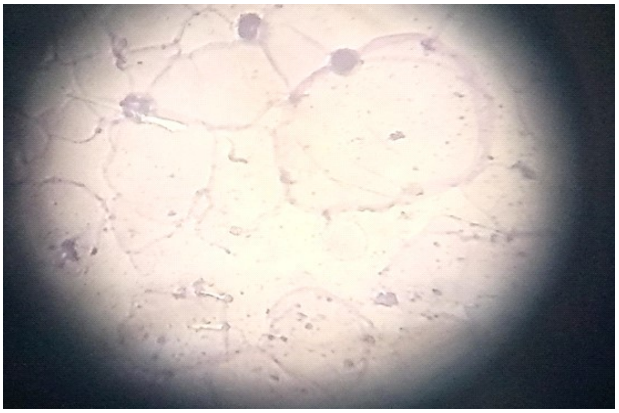

Plate 1: Porcine blood smear infected with *Trypanosoma brucei* (arrowed) using microscopy

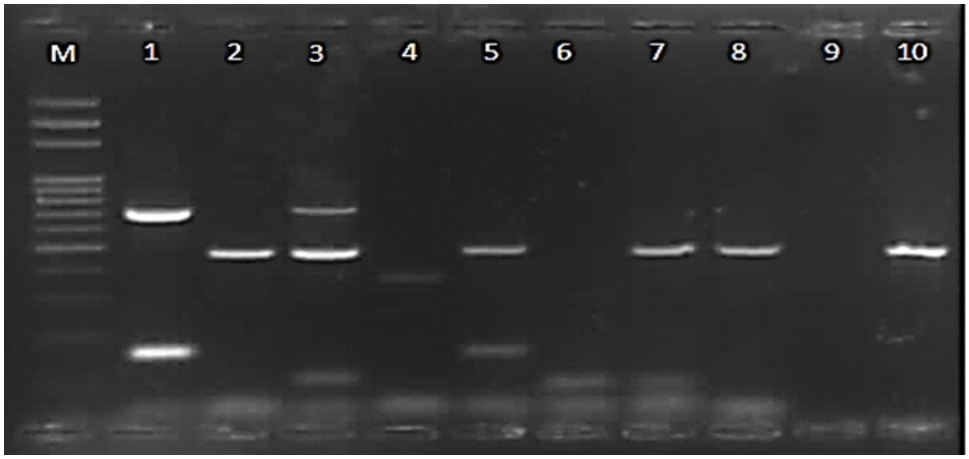

Plate 2: Agarose Gel picture showing the amplicon sizes of trypanosomal ITS-1.

Lane M: Molecular weight 100 bp DNA ladder; Lane 1- *T. congolense* in a Pig from FCT; 2- *T. brucei* in a Pig from FCT; 3- *T. brucei* and *T. congolense* in a Pig from FCT; 4- *T. simae* in a Pig from FCT; 5- *T. brucei* in a Pig from FCT; 6-Negative; 7- *T. brucei* in a Pig from Nasarawa; 8- *T. brucei* in a Pig from FCT; 9-Negative control;10- *T. brucei* positive control

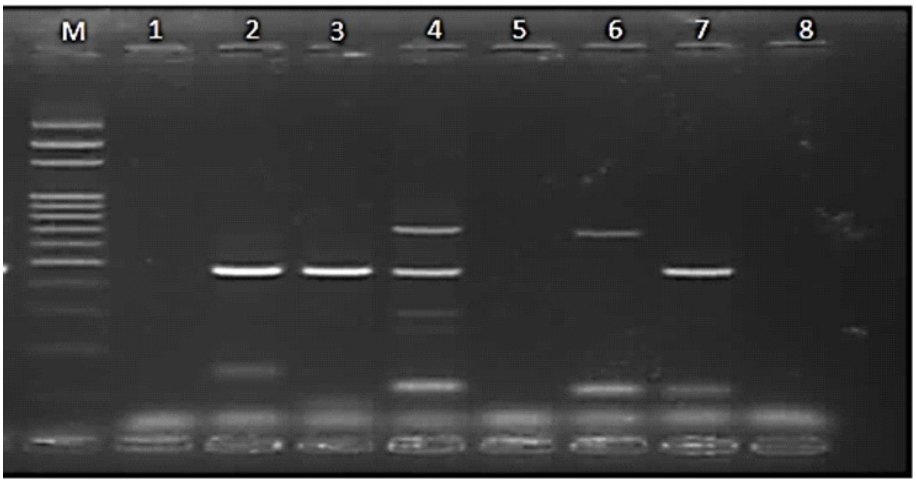

Plate 3: Agarose gel picture showing the amplicon sizes of trypanosomal ITS-1
Lane M: Molecular weight 100 bp DNA ladder; Lane 1- Trypanosome-negative sample (Pig); Lane 2- *T. brucei* in a Pig from FCT; Lane 3- *T. brucei* in a Pig from FCT; Lane 4- *T. brucei, T. vivax*, *T. godfreyi* and *T. congolense* in a Pig from Nasarawa; Lane 5- Trypanosome-negative in Cattle from FCT; Lane 6- *T. congolense* in Cattle from FCT; Lane 7- *T. brucei* in Cattle from FCT; Lane 8- Trypanosome-negative in Cattle from FCT

Table 1: Prevalence of Single and Double *Trypanosoma species* positives in sampled Pigs from Gwagwalada, Kuje and Kwali Area Councils of Federal Capital Territory

	Single Infection												Double Infection		
	T. congo			*T. simiae*			*T. brucei*			*T. evansi*			*T. congo* and *T. brucei*		
	N	P	%	N	P	%	N	P	%	N	P	%	N	P	%
Gwag	50	0	0.00	50	0	0.00	50	3	6.00	50	0	0.00	50	0	0.00
Kuje	38	0	0.00	38	0	0.00	38	0	0.00	38	0	0.00	38	0	0.00
Kwali	97	2	2.06	97	1	1.03	97	7	10.31	97	2	2.06	97	2	2.06

N-Number sampled; P- Number positive; % Prevalence; Gwag- Gwagwalada

Table 2: Prevalence of Single, Double and Quadruple *Trypanosoma species* positives in sampled pigs from Karu, Keffi, and Nasarawa Local Government Areas of Nasarawa State

	Single Infection						Double Infection			Quadruple Infection		
	T. congo			*T. brucei*			*T.* congo and *T. brucei*			*T. congo, T. brucei, T. vivax* and *T.godfreyi*		
	N	P	%	N	P	%	N	P	%	N	P	%
Karu	71	1	3.33	71	3	10.00	71	1	3.33	71	1	3.33
Keffi	63	0	0.00	63	1	7.69	63	0	0.00	63	0	0.00
Nasarawa	76	0	0.00	76	0	0.00	76	1	14.29	76	0	0.00

N-Number sampled; P- Number positive; % Prevalence

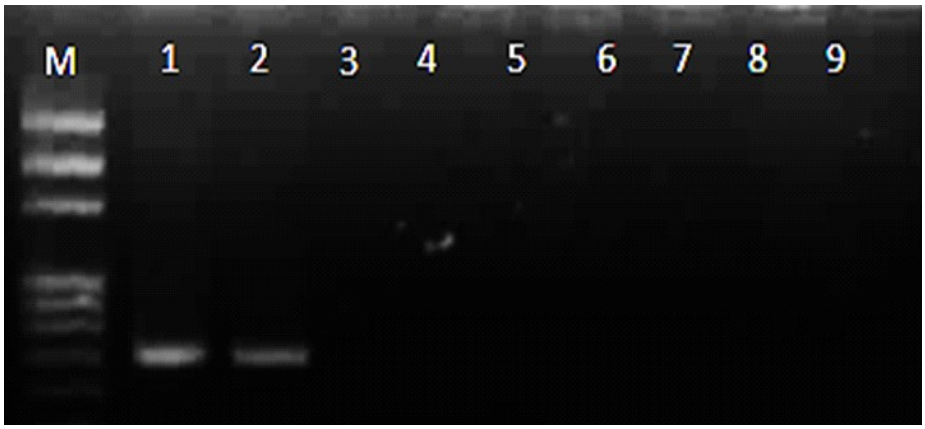

Plate 4: Agarose gel picture showing the amplicon sizes of *T. evansi* positives

Plate 5: 3-months old pigs infected with *Trypanosoma brucei* and *T. congolense* showing signs of generalized alopecia and hyperemia of the skin

Plate 6: Hurdling among weaners infected with *T. brucei* and *T. congolense* in a Pig farm in Federal Capital Territory

Plate 7: Piloerection in a *T. brucei* and *T. congolense* infected weaned piglet in a pig farm in Keffi Local government of Nasarawa State

Table 3: The Observed Clinical signs with prevalence of single *Trypanosoma species* in sampled pig population

CS	TAE	TAP + CS	TA + *T. congo* + CS (%)	TA + *T. congo* – CS (%)	TA + *T. brucei* + CS (%)	TA + *T. brucei* – CS (%)	TA + *T. simae* + CS (%)	TA + *T. simae* - CS (%)
Emaciation	395	25	2(66.7)	1(33.3)	11(78.6)	3(21.4)	0(0.0)	1(100.0)
Pilo erection	395	25	0(0.0)	3(100.0)	10(71.4)	4(28.6)	0(0.0)	1(100.0)
Ataxia	395	25	0(0.0)	3(0.25)	1(7.1)	13(92.9)	0(0.0)	1(100.0)
PMM	395	25	1(33.3)	2(66.7)	2(14.3)	12(85.7)	1(100.0)	0(0.0)
RHC	395	25	0(0.00)	3(100.0)	4(28.6)	10(71.4)	1(100.0)	0(0.0)
HoS	395	25	0(0.00)	3(100.0)	13(92.9)	1(7.1)	0(0.00)	1(100.0)

Key: CS-Clinical sign, PMM-Pale Mucous Membrane, RHC-Rough Hair Coat, HoS- Hyperemia of Skin, TA-Total Animal, TAE- Total Animals Examined, TAP-Total animal positive, *T. congo- Trypanosoma congolense, T. brucei- Trypanosoma brucei, T. simae-Trypanosoma simae*, +-with, - - without

Table 4: The Observed Clinical signs with prevalence of single and mixed double *Trypanosoma species* in sampled pig population

CS	TAE	TAP + CS	TA+ *T. evansi* + CS (%)	TA + *T. evansi* – CS (%)	TA + *T. congo* +*T. brucei* + CS (%)	TA + *T. congo*+*T. brucei* - CS (%)
Emaciation	395	25	1(50.0)	1(50.0)	3(75.0)	1(25.0)
Pilo erection	395	25	0(0.0)	2(100.0)	3(75.0)	1(25.0)
Ataxia	395	25	0(0.0)	2(100.0)	0(0.0)	4(100.0)
PMM	395	25	0(0.0)	2(100.0)	3(75.0)	1(25.0)
RHC	395	25	1(50.0)	1(50.0)	0(0.0)	4(100.0)
HoS	395	25	0(0.0)	2(100.0)	0(0.0)	4(100.0)

Key: CS-Clinical sign, PMM-Pale Mucous Membrane, RHC-Rough Hair Coat, HoS- Hyperemia of Skin, TA-Total Animal, TAE- Total Animals Examined, TAP-Total animal positive, *T. congo- Trypanosoma congolense, T. brucei- Trypanosoma brucei, T. simae-Trypanosoma simae*, +-with, - - without

Table 5: The Observed Clinical signs with prevalence of mixed quadruple *Trypanosoma species* in sampled pig population

CS	TAE	TAP +CS	TA with *T. congo* +*T. brucei* +*T. vivax* +*T.godfreyi* + CS (%)	TA with *T. congo* +*T. brucei* +*T. vivax* +*T.godfreyi* - CS (%)
Emaciation	390	25	1(100.0)	0(0.0)
Pilo erection	390	25	0(0.0)	1(100.0)
Ataxia	390	25	0(0.0)	1(100.0)
PMM	390	25	1(100.0)	0(0.0)
RHC	390	25	1(100.0)	0(0.0)
HoS	390	25	0(0.0)	1(100.0)

Key: CS-Clinical sign, PMM-Pale Mucous Membrane, RHC-Rough Hair Coat, HOS- Hyperemia of Skin, TA-Total Animal, TAE- Total Animals Examined, TAP-Total animal positive, *T. brucei*- *Trypanosoma brucei*, *T.congo*-*Trypanosoma congolense*, *T. vivax*- *Trypanosoma vivax*, *T. godfreyi*- *Trypanosoma godfreyi*, + - with, - - without

Table 6: Mean Haematology of PCR Trypanosome infection in Pigs sampled in Federal Capital Territory and Nasarawa state

	Uninfected	Single Infection				Double Infection	Quadruple infection
Haematological Indices	Uninfected	*T. congolense*	*T. simiae**	*T. brucei*	*T. evansi*	*T.c/T.b*	*T. c/T. b/T. v/T.g**
Packed Cell Volume (%)	43.01±3.21^{d}	31.38±1.22^{b}	35.31±0.00	38.21±4.53^{e}	34.31±0.86^{e}	25.23±1.81^{a}	18.23±0.00
Red blood cell count (X10^{6})	6.33±0.04^{d}	4.27±0.12^{b}	3.85±0.00	5.83±0.56^{c,d}	5.55±0.46^{c}	3.31±0.61^{a}	3.23±0.00
Haemoglobin concentration (mg/dl)	14.22±1.01^{e}	10.19±0.32^{b}	12.6±0.00	12.22±2.10^{e}	14.1±0.61^{e}	8.72±1.09^{a}	5.21±0.00
Mean corpuscular volume (fl)	62.45±3.89^{c}	52.15±2.34^{b}	58.33±0.00	60.03±2.64^{c}	43.33±2.75^{a}	47.73±2.33^{b}	38.23±0.00
Mean corpuscular haemoglobin (pg)	19.94±1.23^{c}	17.41±3.02^{b}	17.22±0.00	17.44±2.33^{b}	14.22±0.08^{a}	15.21±0.24^{b}	10.15±0.00
Mean corpuscular haemoglobin concentration (mg/dl)	30.85±2.10^{b}	33.57±0.36^{c}	28.92±0.00	32.81±2.60^{b}	34.92±1.78^{c}	21.91±0.98^{a}	25.33±0.00
Total white blood cell count (X10^{3})	21.52±1.02^{c}	8.49±0.59^{a}	23.01±0.00	20.50±1.22^{c}	25.01±1.01^{d}	15.65±1.70^{b}	30.12±0.00
Lymphocyte count (%)	48.23±4.86^{b}	35.39±0.48^{a}	40.05±0.00	69.34±9.36^{d}	34.43±2.67^{a}	57.22±1.79^{c}	40.12±0.00
Monocyte count (%)	2.56±0.02	2.51±0.97	2.01±0.00	2.10±0.07	2.01±0.15	2.01±1.80	2.12±0.00
Neutrophil count (%)	51.67±3.24^{d}	34.43±3.52^{a,b}	33.12±0.00	47.70±2.04^{c}	32.46±0.43^{a}	35.20±1.99^{b}	31.11±0.00
Eosinophil count (%)	3.60±0.02	3.05±0.46	3.40±0.00	3.54±0.19	3.50±0.36	3.01±0.97	3.41±0.00
Basophil count (%)	1.02±0.11	1.16±0.11	1.40±0.20	1.01±0.41	1.30±0.15	1.01±0.4	1.45±0.20
Platelet (%)	345.16±13.20^{b}	330.75±15.71^{b}	350.34±0.00	200.12±17.68^{a}	346.34±65.19^{b}	309.00±44.98^{b}	301.31±0.00

Discussion

This study has shown that African Animal Trypanosomosis is still in circulation among livestock despite the several years of control by NITR and PATEEC (Odeniran et al., 2018). In comparison with microscopy, more trypanosomes were identified using ITS-PCR. This is expected because the sensitivity of microscopy is dependent on the stage of infection (very high, early infection; low, chronic infection; nil; healthy carriers and extravasular infection), unlike PCR. The low prevalence with microscopy could also be as a result of poor performance of microscopists in the diagnosis of trypanosomes in North-central Nigeria (Akinbobola et al., 2023a). This result is in agreement with other animal trypanosomiasis epidemiological studies that have shown the superiority of PCR over micro-haematocrit centrifugation technique (Takeet et al., 2013, Habeeb et al., 2021). In pigs,

the overall trypanosome prevalence observed in this study was lower than reports from earlier studies in some areas in Nigeria (Omeke, 1994, Karshima et al., 2016) and Ghana (Nakayima et al., 2012, Apatah et al., 2020). Factors that could be responsible for these variations are seasons, production systems, human activity which include the rational use of trypanocidal drugs as well as the migration of cattle and occurrence of tsetse flies as earlier observed (Majekodunmi et al., 2013). Overall, there was a predominance of *T. brucei* over other trypanosome species. In agreement with the report of Karshima et al. (2016), this could be as a result of *Trypanosoma brucei* preference for porcine species, suggesting that the population of trypanosome in any ecosystem is dependent on different host and vector interaction. However, this predominance in cattle could result from the resistance of *T. brucei* parasites to diminazene and isometamidium drugs which are commonly used in the management of bovine trypanosomosis (Giordani et al., 2016, Kasozi et al., 2022). Although, mixed infection involving three trypanosoma species which are *T. congolense, T. vivax and T. brucei* has been reported (Mwandiringana et al., 2012, Odeniran et al., 2018), this study is the first to report quadruple mixed infections involving *T. congolense*, *T. brucei* s. l*, T. vivax and T. godfreyi* in a pig from Karu local government area of Nasarawa state. This quadruple infection could occur because a tseste fly has different trypanosome development predilection sites, thus allowing for the transmission of one or more than one trypanosome species from an infected host to another (Simwango et al., 2017). The implication of this diversity of single and mixed Trypanosomes in pigs is that just as pigs are sequentially infected on the field, they can also serve as reservoirs of *Trypanosoma species* that are pathogenic to other livestock. Our study found no evidence of *T. brucei gambiense* in pigs using TgsGP PCR and immune trypanolysis, indicating that it is unlikely either cattle or pigs are acting as cryptic reservoirs of disease. This could be because Gambiense HAT has a near zero prevalence in Nigeria. This result agrees with reports from previous studies in pigs from Taraba states (Karshima et al., 2016), Livestock from Niger state (Enwezor et al., 2019) and Uganda (Cunningham et al., 2020). It is noteworthy that trypanosomes were earlier reported to reside in the skin and adipose tissue of animal host (Capewell et al., 2016). As a result, the skin has been proposed to be an anatomical reservoir for transmission (Alfituri et al., 2020). Thus, with the availability of diagnostic toolbox, the skin could be adopted as diagnostic sites of *T. b. gambiense* positive animals.

In pigs, extravascular clinical abnormalities (hyperemia and pilo-erection) in *T. brucei* single and mixed *Trypanosoma species* infection could result from the capability of *T. brucei* to pass out of the capillaries into the interstitial tissues and serous fluids of body cavities where they continue to multiply and damage tissues (Luckins et al., 1994). These observed clinical signs are at variation with the report of Onah (1991), who reported anaemia, emaciation and anestrus as the most important effects associated with porcine trypanosomosis. These variations in clinical outcomes could be as a result of concurrent infection associated with field studies or complex interaction between the host, parasite and environment (Akinbobola et al., 2023a, Akinbobola et al., 2023b).

In comparison with single, double infections and uninfected groups, severe haematological changes was observed with mixed quadruple trypanosome infection in infected pig. This could be due to a combination of exaggerated divers mechanisms of pathology which is partly in relation to the localization of the *Trypanosoma species* involved, causing damage both intravascularly (*T. congolense*) and within tissue and organ (*T. brucei*). To the best of our knowledge, this is the first report documenting the haematology of quadruple

trypanosomes infection in a pig. Our study also reported leucopenia due to neutropenia and lymphotopenia in *T.congolense* and *T. vivax* natural infection in cattle. This could arise as a result of massive peripheral utilization, phagocytosis in the bone marrow and other organs such as liver and spleen and transformation of leucocyte to plasma cell which occur during infection (Abenga et al., 2005). This report is in agreement with the report of Ganyo *et al.* (2018) that natural *T. vivax* infection in cattle resulted in leucopenia. Although pigs were infected with other *Trypanosoma species*, we found no evidence that pigs in FCT and Nasarawa state are acting as reservoir hosts of *T. b. gambiense* at the time of study.

Conclusions

At the time of study and in the study location, pigs are reservoirs of trypanosome species that are pathogenic to other animal species but not humans. Clinicians should also acquaint themselves with current trend of clinical signs to avoid speculative diagnosis which potentiates drug resistance.

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