

# ORIGINAL RESEARCH ARTICLE

# **Detection and Epidemiology of Trypanosome Infection in Livestock at Katsina Abattoir: Implications for Biosecurity and Animal Health in Northern Nigeria**

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#### **ABSTRACT**

African Animal Trypanosomiasis, a vector-borne parasitic disease caused by Trypanosoma species and transmitted by tsetse flies, severely impacts livestock health and agricultural productivity across sub-Saharan Africa. This study investigates the prevalence and impact of trypanosome infections in livestock slaughtered at Katsina Central Abattoir Katsina State, Nigeria, focusing on species circulating in the region, which is traditionally outside the Tsetse fly belt. Despite the absence of tsetse flies, mechanical transmission by biting insects and crossborder livestock movement from endemic areas pose significant risks. We examined 200 animals, including goats, sheep, cattle, and camels, categorizing 146 as healthy and 54 as nonhealthy based on body condition and packed cell volume (PCV). Non-healthy animals exhibited significantly lower PCV values, correlating with trypanosome infections. Microscopy identified trypanosome parasites in one goat, one sheep, and one camel, all from the non-healthy group. Molecular analysis using (ITS-1 PCR) further confirmed the presence of *Trypanosoma evansi* in the camel. These findings present the need for enhanced diagnostic techniques in routine veterinary practices and robust biosecurity measures such as quarantining newly introduced livestock, public awareness about the risks associated with livestock movement, and informed policy decisions to mitigate the spread of trypanosomiasis, particularly in regions exposed to crossborder livestock movement. This research promotes a proactive approach to managing emerging zoonotic diseases in vulnerable regions. Strengthening surveillance and implementing targeted control strategies are essential to minimizing the economic burden of trypanosomiasis on Nigeria's livestock industry, particularly and on Africa as a whole.

# **INTRODUCTION**

Trypanosomes are unicellular parasitic flagellates of the order Kinetoplastida, primarily transmitted through the bites of various *Glossina* species, commonly known as tsetse flies. Non-tsetse transmitted trypanosomes, such as *Trypanosoma evansi* (*T. evansi*) and *Trypanosoma equiperdium* (*T. equiperdium*), along with tsetse-transmitted species like *Trypanosoma brucei* (*T. brucei*), *Trypanosoma congolense* (*T. congolense*), and *Trypanosoma vivax* (*T. vivax*) (which can be transmitted by both tsetse and non-tsetse flies), have significantly restricted livestock farming across vast regions [\(Giordani](#page-7-0) *et al.,* 2016; [Hargrove](#page-7-1) *et al.,* 2012). Infection by one or more of these trypanosome species results in acute or chronic disease, often referred to as African trypanosomiasis (AT), characterized by symptoms such as emaciation, anaemia, loss of appetite, weakness, corneal opacity, occasional diarrhoea, parasitaemia,

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infertility, abortion, coma, and, if untreated, death [\(Brutus](#page-6-0)  *[et al.,](#page-6-0)* 2010; [Underwood](#page-9-0) *et al.,* 2015). The clinical manifestation of trypanosomiasis in animals is influenced by both the host and the trypanosome species and strain [\(Behour](#page-6-1) *et al.,* 2019). Animals that survive infection often remain carriers for several months or years, exhibiting low-level fluctuating parasitemia, serving as a reservoir for the disease. Notably, resistance to current treatments has also been reported [\(Magez](#page-8-0) *et al.,* 2008; [Trindade](#page-9-1) *et al.,* [2016\)](#page-9-1). These collective effects typically lead to reduced reproduction rates, lower feed conversion ratios, and increased mortality, all of which significantly impact farmers' profits [\(Machina](#page-8-1) *et al.,* 2017).

Domestic sheep and goats, integral to pastoral and agropastoral livestock production, are particularly important as

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they provide over 30% of local meat consumption and are vital sources of income for small-scale farmers [\(Montossi](#page-8-2)  *et al.,* [2013\)](#page-8-2). Trypanosomiasis infection in sheep and goats is frequently reported, with substantial economic impacts on small ruminants [\(Njuki](#page-8-3) *et al.,* 2009). Camel trypanosomiasis is also part of the leading causes of morbidity and mortality in camels in many parts of Africa. It causes anorexia, weakness, and emaciation, leading to reduced milk and meat yields, poor traction power, increased abortion rates, and death. Treatment costs further burden camel breeders and the national economy [\(Tekle & Abebe, 2001\)](#page-9-2). Cattle are another critical component of pastoral and agro-pastoral systems, contributing significantly to both meat production and income, with over 40% of local meat consumption relying on them (Kim *[et al.,](#page-8-4)* 2017; [Ochungo](#page-9-3) *et al.,* 2019). Trypanosomiasis in cattle results in severe economic losses due to reduced milk and meat production, increased mortality rates, and high treatment costs [\(Fadiga](#page-7-2) *et al.,* [2011\)](#page-7-2). Thus, trypanosomiasis is a significant livestock disease in Africa, posing a threat to poverty alleviation efforts across the continent.

In Nigeria, livestock rearing remains a vital occupation, with the livestock value chain meeting various human needs. However, African Animal Trypanosomiasis (AAT) poses a significant threat to its economic potential. Previously tsetse-free or reclaimed areas are now being reinvaded by tsetse flies [\(Isaac](#page-7-3) *et al.,* 2017). The rise in human population and subsequent increase in human activities have led to significant changes in the availability of suitable habitats and hosts, ensuring the survival and persistence of trypanosome vectors [\(Dede](#page-7-4) *et al.,* 2005). In Nigeria, non-tsetse transmitted trypanosomiasis has a prevalence of approximately 27%, higher than in some other AAT-endemic countries like Mauritania (24%), Ethiopia (21%), and India (22%). Various ecological factors, including temperature, climate, rainfall, and vegetation type, influence the distribution of tsetse flies. Extreme temperatures are unfavourable for their activity and infectivity [\(Are & Hargrove, 2020;](#page-6-2) [Nnko](#page-9-4) *et al.,* 2017). The trypanosome vector thrives in regions receiving over 1,000 mm of rainfall [\(De Meeûs](#page-7-5) *et al.,* 2012). Vegetation, such as dense forests, bushy lands, and savanna grasslands, provides ideal habitats that protect tsetse flies from environmental stressors like sunlight and wind [\(Cecchi](#page-7-6) *et al.,* [2008\)](#page-7-6). In Nigeria, trypanosome infection has been reported with prevalence rates of 36.8% and 16.6% in the North-Central and North-East zones, respectively [\(Karshima](#page-8-5) *et al.,* 2016). A study in Benue state found a prevalence of 41.7% among 163 screened animals [\(Omotainse](#page-9-5) *et al.,* 2000). In Plateau state, a 12.2% prevalence was observed among 740 animals [\(Kalajaiye](#page-7-7) *et al.,* [2004\)](#page-7-7), while Bauchi state reported a 51% prevalence among 448 animals [\(Obaloto](#page-9-6) *et al.,* 2015). In Kaduna state, a prevalence of 2.1% was recorded among 529 animals screened [\(Samdi](#page-9-7) *et al.,* 2010). A more recent study in 2017 found a prevalence of 40.9% among 110 animals [\(Benjamin](#page-6-3) *et al.,* 2017). A collective study across Kaduna, Kano, and Sokoto states reported a prevalence of 75.0%

among 50 animals [\(Sanni](#page-9-8) *et al.,* 2013). In Sokoto State, an observational report on cattle discovered 9 positive samples, corresponding to a prevalence of 1.8% (Fajinmi *et al.,* [2007\)](#page-7-8). Additionally, the prevalence of trypanosome infections reported across various abattoirs in Northern Nigeria continues to highlight significant public health and economic concerns. Studies reveal infection rates ranging from 1.5% to 21.3% among cattle and camels, with species such as *T. brucei*, *T. congolense*, and *T. vivax* identified as predominant pathogens [\(Enwezor](#page-7-9) *et al.,* 2023; [Lema](#page-8-6) *et al.,* [2018;](#page-8-6) [Mohammad](#page-8-7) *et al.,* 2021), while factors such as sex, breed, and age appear to influence infection prevalence, with female cattle and certain breeds, like the Adamawa Gudali, showing higher susceptibility [\(Chibuogwu](#page-7-10) *et al.,* [2023;](#page-7-10) [Muhammad](#page-8-8) *et al.,* 2023). Molecular techniques like PCR enhance detection sensitivity compared to traditional microscopy, indicating the need for integrated diagnostic approaches to effectively manage and control trypanosomiasis (Isa *et al.,* [2020;](#page-7-11) [Kamani](#page-8-9) *et al.,* 2022).

Katsina, while typically outside the tsetse fly belt, faces a heightened risk of non-tsetse transmitted trypanosomiasis due to cross-border livestock movement from trypanosome-endemic regions, highlighting the importance of updated surveillance strategies [\(Hargrove](#page-7-1) *et al.,* [2012;](#page-7-1) [Isaac](#page-7-3) *et al.,* 2017). The outbreaks in northern Nigeria, particularly in states neighbouring Katsina, and the reinvasion of previously tsetse-free areas driven by population growth and changes that create suitable habitats for disease vectors emphasize the urgent need for further investigation into animal trypanosome infections [\(Are & Hargrove, 2020;](#page-6-2) [Isaac](#page-7-3) *et al.,* 2017; [Machina](#page-8-1) *et al.,* [2017\)](#page-8-1). This study was conducted to detect the presence of trypanosome infection in animals slaughtered at the Katsina abattoir in Katsina State, Nigeria, using a combination of microscopy and molecular-based (PCR) diagnosis. Additionally, we evaluated the association between packed cell volume (PCV) and trypanosome infection in the screened.

# **MATERIALS AND METHODS**

#### **Study site and sample collection**

This study was conducted at the Katsina Central Abattoir, Katsina L.G.A., Katsina State, Nigeria, from September 2019 to January 2020. To determine the appropriate sample size, we used the formula for estimating proportions with a 95% confidence interval and a 5% margin of error, assuming a prevalence rate of 50% for trypanosomiasis in the local animal population. This calculation resulted in a sample size of 200 animals, including cattle, camels, sheep, and goats [\(Figure 1\)](#page-2-0). Ethical approval for this study was granted by the Ethics Committee of Umaru Musa Yar'adua University Katsina (UMYUK) prior to the commencement of sample collection. The animals were assessed for health status based on their physical condition. Animals included in the study were those older than 6 months that presented no evidence of infection. Animals showing signs of disease, younger than 6 months, and animals not intended for

slaughter at the abattoir were excluded. From each animal, 1 mL of blood was aseptically collected at the time of slaughter and mixed with Ethylenediaminetetraacetic Acid

(EDTA) in labelled bottles. The samples were then transported in ice-packed boxes to the Microbiology Laboratory at UMYUK Katsina for analysis.

<span id="page-2-0"></span>

**Figure 1: Geographical representation of Katsina State, Nigeria, highlighting Mai'adua and Charanchi markets.**  (A) Nigeria (in blue) within Africa, (B) the location of Katsina state (in red) on the Nigerian map, (C) the location of Mai'adua and Charanchi Local Government Areas within Katsina state (in red) as the main sites where animals slaughtered at Katsina abattoir are sourced.

# **Determination of packed cell volume**

All blood samples were analysed in triplicate using the method described by [\(Simukoko](#page-9-9) *et al.,* 2007) without modification. Briefly, samples from each EDTA bottle were transferred into capillary tubes, sealed at one end with plasticine, and centrifuged at 9,000 rpm for 5 minutes in a microhematocrit centrifuge. PCV was then determined using a microhematocrit reader.

#### **Parasitological examination**

The Wet Blood Film (WBF) technique was used to detect trypanosomes. A drop of blood was placed on a microscope slide, covered with a  $22 \times 22$  mm coverslip, and lightly pressed to spread the blood. Approximately 50–100 fields per slide were examined at ×400 magnification, with the light condenser lowered to enhance the visibility of the plasma membranes. Blood smears were further prepared, air-dried, fixed in absolute methyl alcohol for 3 minutes, and stained with Giemsa's stain. Stained smears were examined under an oil immersion lens (×1000) to confirm trypanosome presence. Approximately 50–100 fields were examined before a sample was considered negative. If trypanosomes were detected, an additional 20 fields were analysed to check for multiple species [\(Benjamin, 1986\)](#page-6-4). Trypanosomes were identified by their characteristic movement and flagellar structure.

### **Molecular identification**

Microscopically positive samples were preserved on filter paper and sent to the Nigerian Institute for Trypanosomiasis Research (NITR) in Kaduna for molecular characterization. The filter papers were chopped into pieces, placed in Eppendorf tubes, and treated with 200 µL lysis buffer, 20 µL proteinase K, and 10 µL RNAse. DNA was extracted using a DNA extraction mini kit (Qiagen, UK) according to the manufacturer's instructions. DNA concentration and purity were measured using a NanoDrop spectrophotometer and stored at -20 °C.

A nested PCR was performed using Internal Transcribed Spacer 1 (*ITS1*) primers (*ITS1* CF – 5'-3': CCGGAAGTTCACCGATATTG and ITS1 BR – 5'-3': TTGCTGCGTTCTTCAACGAA) in a 50-µL reaction mixture containing  $10\times$  reaction buffer, 2 mM MgCl<sub>2</sub>, 200 µM dNTPs, 1 µM primers, and 0.5 U Taq DNA

polymerase (Inqaba biotec, Zymo Research). All reagents were of high-quality standard and handled in a dedicated PCR workspace to minimize contamination risks. The PCR cycles included initial denaturation at 95 °C for 5 minutes, followed by 34 cycles of 94 °C for 1 minute, 46 °C for 45 seconds, and 72 °C for 1 minute, with a final extension at 72 °C for 10 minutes. The second-round PCR used the amplified DNA from the first round as the template, with the annealing temperature reduced to 40 °C [\(Njiru](#page-8-10) *et al.,* 2005). A negative control was included by using the PCR reagents without DNA to detect any potential contamination. Amplification products were resolved on a 1% agarose gel stained with ethidium bromide, run at 80 V for 1 hour, and visualized under UV light*.*

# **Statistical analysis**

The prevalence of infection was calculated by dividing the number of infected animals by the total number of animals examined, expressed as a percentage. PCV was calculated to assess anaemia levels, expressed as mean ± standard deviation. Statistical significance was evaluated using Student's t-test and one-way analysis of variance using Microsoft Excel (Office 2021).

# **RESULTS AND DISCUSSION**

A total of 200 animals were screened and classified based on their physical condition into healthy and non-healthy groups [\(Table 1\)](#page-3-0) with the aim of identifying the anaemic conditions of livestock, including goats, sheep, cattle, and camels, slaughtered at Katsina abattoir–a region

traditionally outside the tsetse belt, which is a key indicator of trypanosome infections. The healthy-looking animals demonstrated a mean PCV of  $31.09 \pm 4.14\%$ , while the non-healthy animals exhibited a significantly lower mean PCV of  $27.11 \pm 5.94\%$ . The lower PCV in non-healthy animals is indicative of anaemia, a condition commonly associated with trypanosome and parasitic infections. Although there is an absence of tsetse flies in the study area, mechanical transmission by hematophagous insects such as tabanids and Stomoxys flies remains a significant concern for species like *T. vivax* and *T. evansi*. Additionally, *T. equiperdium* could be sexually transmitted [\(Desquesnes](#page-7-12)  [& Dia, 2003;](#page-7-12) [Giro](#page-7-13) *et al.,* 2020; Lai *et al.,* [2008\)](#page-8-11). Therefore, there is need for vigilant surveillance even in regions outside the traditional tsetse belt. The introduction of animals from trypanosome-endemic areas, including neighbouring Niger Republic and other Nigerian states, further exacerbates the risk of spreading trypanosomiasis within Katsina State. Markets such as Mai'adua and Charanchi serve as major livestock trading hubs, facilitating the movement of potentially infected animals into the state [\(Isaac](#page-7-3) *et al.,* 2017). Furthermore, increased activities of kidnappers, bandits, and cattle rustlers in the region have forced non-pastoralists to congest more animals in one place and forced pastoralists to adopt alternative, often riskier routes, which may further elevate the transmission of trypanosomiasis and other diseases. These security challenges also hinder regular veterinary checks and disease control measures, increasing the vulnerability of livestock to infections [\(Aruwayo](#page-6-5) *et al.,* [2021\)](#page-6-5).

<span id="page-3-0"></span>**Table 1: Mean packed cell volume (PCV) of healthy and non-healthy looking animals slaughtered at Katsina abattoir.**

Sample	No. examined	Mean PCV $(\% )$
Healthy looking animals	!46	$31.09 \pm 4.14$
Non-healthy looking animals		27 11 + 5 94
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Data were analysed using Student's t-Test;  $p \le 0.05$ .

The correlation between low PCV and other parasitic infections, including those caused by *Moniezia spp.*, *Babesia motasi*, *Babesia ovis*, *Anaplasma ovis*, *Anaplasma marginale*, and *Eperythrozoon ovis*, is well-documented [\(Lelisa &](#page-8-12)  [Meharenet, 2021\)](#page-8-12). Therefore, chronic parasitic infections and infestations by fleas, lice, and ticks, as well as gastrointestinal nematodes, can further contribute to the anaemia observed in these animals [\(Villanueva-Saz](#page-9-10) *et al.,* [2022;](#page-9-10) [Walden](#page-9-11) *et al.,* 2022).

During this study, the abattoir primarily sourced its slaughtered animals from the Mai'adua (35%) and Charanchi (40%) markets. When the animals were further categorised based on their origin, significant variations in PCV levels were observed [\(Table 2\)](#page-4-0). A one-way analysis of variance shows significant differences in PCV across different animal species and reveals noteworthy trends. Notably, animals from Mai'adua consistently show lower mean PCV values compared to their counterparts from other origins. Typically, PCV values in goats, sheep, cattle, and camels range between 22-38%, 27-45%, 24-46%, and

24-35% respectively [\(Al-Bulushi](#page-6-6) *et al.,* 2017; [Feldman](#page-7-14) *et al.,* [2000;](#page-7-14) [Jackson & Cockcroft, 2002\)](#page-7-15). The mean PCV of goats from Mai'adua was  $20.38\% \pm 6.60$ , which is considerably lower than the values observed in Charanchi  $(32.17\% \pm 5.15)$  and goats from an unknown location  $(32.82\% \pm 5.21)$ . Conversely, similar PCV values in both Katsina (29.83%  $\pm$  4.80) and Mai'adua were observed, reiterating that goats from these regions might be under similar adverse conditions, including the risk of infection. For sheep, the mean PCV in Mai'adua was  $25.08\% \pm 6.74$ , the lowest among the examined sources. In contrast, sheep from Charanchi (30.70%  $\pm$  6.31) and Katsina  $(32.17\% \pm 6.85)$  exhibit significantly higher values. This notable difference also indicates a potential health issue affecting sheep in Mai'adua, possibly linked to factors such as management practices and/or disease prevalence, including trypanosomiasis.

When comparing cattle, those from Mai'adua had a mean PCV of  $30.33\% \pm 6.38$ , again lower than the values recorded in animals from unknown location (31.50%) and

indicating a concerning trend. Charanchi (29.28%  $\pm$  3.80) and Katsina (29.79%  $\pm$  4.96) showed closely related PCV values. The camels from Mai'adua also demonstrated low PCV (19.36%  $\pm$  8.63) compared to animals in Charanchi (39.14%  $\pm$  4.37). This low PCV indicates that even camels, which typically have high resilience and adaptability, are suffering from possible silent parasitic infections in addition to limited access to veterinary care. Our analysis highlights a concerning trend of low PCV values below the normal PCV cutoff in animals from Mai'adua across all species examined except for cattle, warranting further investigations and the need for improved management practices [\(Elitok & Cirak, 2018;](#page-7-16) Mannir *et al.,* 2024). Understanding the underlying factors contributing to these low PCV values is important for implementing effective interventions to enhance the welfare of these animals. These findings further emphasize the importance of targeted screening and treatment protocols. The markedly lower PCV in animals from the Mai'adua market may likely reflect the influence of cross-border animal trade with the Niger Republic, where trypanosome is rarely investigated. This could additionally be traced back to historical influence. The severe Sahelian droughts of the 1970s and 1980s resulted

in the loss of 300,000 livestock and a 60% reduction in agricultural output in Northern Nigeria [\(Kalu, 1996\)](#page-8-13). The droughts caused the loss of 5 million tonnes of grain, 120,000 livestock due to conflict, and forced migrations and displacement that further strained the introduction and spread of tsetse flies and trypanosomiasis [\(Nyong,](#page-9-12)  [2001\)](#page-9-12). Pastoralists across the Republic of Niger and Northern Nigeria adopted migration to Southern Nigeria as a coping strategy, where they remained for extended periods before returning northward [\(Tatard](#page-9-13) *et al.,* 2017). This mass movement of animals likely facilitated the spread of trypanosomiasis in the north, including Katsina, a key transit route along the West African cattle corridor from the Niger Republic [\(Kasozi](#page-8-14) *et al.,* 2021; [Mannir](#page-8-15) *et al.,* [2024\)](#page-8-15). The low PCV levels observed in animals at the Katsina abattoir may also be an indication of the silent impact of non-AAT infection in this region. The clinical signs of other infections, including anaemia, are consistent with the findings from this study. Therefore, these findings suggest addressing this gap through targeted research in Katsina as an essential tool for developing effective control strategies and improving livestock health and agricultural productivity.



<span id="page-4-0"></span>

Notes: Data were analysed using One Way Analysis of Variance (ANOVA), and \* indicates statistical differences (*p* < 0.05).

Katsina Goats 12 29.83±4.80

Others Goats 61 32.82±5.21

Cattle 18 29.28±3.80 Camels 27 39.14±4.36

Sheep 6 32.17±6.85 Cattle 29.79±4.96 Camels 3 33.33±7.57

Sheep 9 29.00 $\pm$ 5.87 Cattle  $1$  31.50 $\pm$ N/A Camels 0 0

Microscopic examination revealed the presence of trypanosome parasites in three animals: one goat (1.58%), one sheep (2.70%), and one camel (1.70%) [\(Table 3\)](#page-5-0). All positive samples were from non-healthy animals with low PCV values, consistent with previous studies linking trypanosome infection to anaemia [\(Marcotty](#page-8-16) *et al.,* 2008). The relatively low prevalence rate of trypanosome infection detected microscopically could be attributed to the transient nature of parasitaemia, particularly in cases where the infection is chronic or subclinical, leading to fluctuating parasite loads that are difficult to detect by microscopy alone [\(Lythgoe](#page-8-17) *et al.,* 2007; [Osaro](#page-9-14) *et al.,* 2014).

To further identify the species detected, molecular diagnostics were employed on the microscopically positive

samples. PCR amplification *ITS-1* primer confirmed the presence of *T. evansi* infection only in the camel sample [\(Figure 2\)](#page-5-1), while the goat and sheep samples did not yield any amplicons [\(Salim](#page-9-15) *et al.,* 2011, [2018\)](#page-9-16). The *ITS-1* primer has become a valuable tool in the identification of various *Trypanosoma* species due to its ability to target an isoform gene that is unique to each detectable species. Recent advancements in molecular diagnostics have further solidified the utility of *ITS-1* primers in distinguishing between *Trypanosoma* species based on the size of their PCR products. The length of *ITS-1* PCR products varies across species, with *T. congolense* savannah producing a 700 bp fragment, *Trypanosoma simiae* a 400 bp fragment, *T. vivax* a 250 bp fragment, and *T. evansi* a 480 bp fragment. Notably, the 480 bp *ITS-1* DNA fragment for detecting *T.* 

*evansi* closely mirrors the DNA fragment length found in *T. brucei* subspecies. This similarity underscores the importance of precise molecular techniques to differentiate between closely related trypanosome species, particularly in regions where multiple species may coexist, complicating diagnosis and treatment strategies [\(Desquesnes](#page-7-17) *et al.,* 2013; [Salim](#page-9-16) *et al.,* 2018). Access to advanced diagnostic tools in Nigeria remains limited, highlighting the ongoing challenges and the critical need for more accessible and robust diagnostic methods in resource-limited settings [\(Isah](#page-7-18) *et al.,* 2024). Recent studies

have also explored the potential for next-generation sequencing and CRISPR-based approaches to enhance the specificity and sensitivity of *ITS-1*-based detection, which is furthering the advancement of accurate trypanosome diagnosis [\(Sabalette](#page-9-17) *et al.,* 2024; [Weisert](#page-10-0) *et al.,* 2024). Our investigation's limitation could also be attributed to factors such as sample contamination, nucleic acid degradation during transport or storage, the presence of PCR inhibitors, or errors during laboratory processing that could contribute to false-negative results [\(Njiru](#page-8-18) *et al.,* [2008\)](#page-8-18).

<span id="page-5-0"></span>



<span id="page-5-1"></span>

# **Figure 2: Molecular identification of trypanosomes in camel sample**

PCR amplification for camel, cattle, goat, and sheep samples. The displayed circled band indicates the positive amplification observed in the camel sample, confirming the presence of trypanosomes. No amplicons were detected in the cattle, goat, and sheep samples, suggesting potential factors such as low parasitaemia or sample contamination.

Our investigation underscores the need for enhanced surveillance and biosecurity measures in livestock markets, particularly in regions with significant cross-border animal trade. Implementing mandatory screening protocols for trypanosomiasis and other parasitic diseases in livestock entering Katsina from high-risk areas could help mitigate the spread of these infections, thereby safeguarding animal health and reducing economic losses associated with trypanosomiasis. The low PCV recorded in the nonhealthy animals, particularly those sourced from high-risk markets such as Mai'adua, not only signals the potential silent impact of AAT but also suggests the presence of other underlying infections that may not have been the primary focus of this study. Anaemia, as indicated by low PCV, is a common manifestation in various parasitic infections beyond trypanosomiasis, including those caused by gastrointestinal nematodes, tick-borne pathogens, and other hemoparasites. Studies have demonstrated that infections by *Besnoitia* spp., *Babesia* spp., *Anaplasma* spp., and *Theileria* spp. can lead to significant reductions in PCV, as these parasites directly or indirectly destroy red blood cells [\(Diezma-Díaz](#page-7-19) *et al.,* 2020).

Additionally, chronic infections with gastrointestinal nematodes such as *Eimeria* spp., *Haemonchus contortus*, *Trichostrongyle*, *Oesophagostomum* spp., *Bunostomum* spp., *Moniezia expansa*, *Trichuris* spp., and mixed infections have been well-documented to cause significant blood loss, leading to anaemia and further compounding the reduction in PCV [\(Hurisa](#page-7-20) *et al.,* 2021). The presence of low PCV in the absence of detectable trypanosome infection in some animals suggests that these other parasitic or bacterial infections could be contributing to the overall health burden in the livestock population of Katsina State. These findings align with the broader literature, which underscores the complexity of anaemia aetiology in livestock, particularly in regions where multiple parasitic infections are endemic [\(Sabatini](#page-9-18) *et al.,* 2023). In light of these, it is imperative that future investigations in Katsina not only focus on AAT but also adopt a more holistic approach, incorporating diagnostic testing for other hemoparasites and gastrointestinal parasites. Such an approach would provide a more comprehensive understanding of the factors contributing to anaemia and other health issues in livestock, ultimately leading to better-informed strategies for disease control and management [\(Oliveira](#page-9-19) *et al.,* 2018). Enhanced surveillance and multi-pathogen screening protocols should be integrated into routine veterinary practice to ensure that the full spectrum of infectious diseases impacting livestock health is adequately addressed [\(Nardini](#page-8-19) *et al.,* [2021\)](#page-8-19).

# **CONCLUSION**

This study highlights the silent yet significant impact of AAT on livestock in Katsina State, Nigeria, particularly in animals sourced from the Mai'adua market, which showed notably low PCV levels–an indicative of anaemia and potential trypanosome or other parasitic infection. This study lays a foundation and presents the need for

comprehensive interventions to mitigate the spread of AAT at the same time considering other possible parasitic, bacterial, and/or viral infections contributing to anaemia. Additionally, the ongoing security threats and challenges endanger livestock and herders, complicating disease control efforts and disrupting veterinary care. We, therefore, suggest necessary screening and quarantine protocols at cross-border livestock trading hubs such as Mai'adua, combined with routine molecular diagnostics, to effectively curb the spread of trypanosomiasis and ensure the health of livestock in Northern Nigeria. These factors require a collective effort, including training veterinary personnel, collaborating with research institutions and security personnel, and creating public enlightenment and awareness to enhance the delivery of veterinary services. Addressing these challenges will ultimately improve livestock health towards sustainable agricultural products.

# **FUNDING STATEMENT**

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