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Interactions among *Sodalis, Glossina pallidipes* salivary gland hypertrophy virus and trypanosomes in wild *Glossina pallidipes*

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Abstract

The successful implementation of area-wide integrated tsetse control using the Sterile Insect Technique (SIT) relies on various factors. These include adapting and mass-producing the wild strain in the laboratory, suppressing the wild population in the target area, and releasing competent sterile male flies into the targeted area. Two important factors that can influence the effectiveness of this strategy are the presence of tsetse endosymbionts, specifically Sodalis glossinidius, and the infection of salivary gland hypertrophy virus (SGHV). SGHV infection directly hampers the expansion of Glossina pallidipes colonies in mass-rearing facilities by negatively impacting reproduction (fertility and fecundity), and overall growth. The viral infections in other tsetse species indirectly affect G. pallidipes by serving as a source of infection, as these species are less susceptible to clinical manifestations of the virus. The role of S. glossinidius in this context remains inconclusive despite previous laboratory and field studies, highlighting the need for further research. In addition to knowledge generation, it is crucial to introduce healthy fly populations from the wild into the insectary for sustainable mass-rearing production. In this study, our objective was to determine the prevalence of Sodalis, SGHV, and trypanosome infections in wild populations of tsetse fly G. pallidipes and investigate potential interactions among them. We analyzed 146 dissected midgut and mouthparts samples from non-teneral flies collected from the Makao Wildlife Management Area (WMA) interface. Our results revealed a prevalence of trypanosome infection at 12.3%, SGHV infection at 7.5%, and Sodalis at 51.4%. Co- existence of SGHV infection and Sodalis was observed in 9.6% of the flies. We did not find a statistically significant association of trypanosome infections occurrence with either SGHV infection or Sodalis. However, a significant association with trypanosome infection was observed when the both Sodalis and SGHV infection co-existed in the tsetse flies. Furthermore, we identified a negative correlation between Sodalis and SGHV infections. In conclusion, our study highlights the association between the co- existence of Sodalis and SGHV and the prevalence of trypanosome infections in wild populations of G. pallidipes. These findings contribute to our understanding of the interactions among these microbes, which is crucial for the development and implementation of effective tsetse control strategies using the Sterile Insect Technique (SIT).

Keywords Sodalis · Mass rearing · Salivary gland hypertrophy · Trypanosomes · Tanzania

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Introduction

The co- existence of symbiotic bacteria, trypanosomes, and other microorganisms in the tsetse fly plays a vital role in the fly's survival and its ability to transmit diseases (Hu et al. 2008; Van Den Abbeele et al. 2010). These microbiotas have diverse interactions within the fly, ranging from beneficial to harmful effects. They can enhance the fly's immunity and reproductive abilities, but they can also induce sterility, impair reproduction, and support the growth of trypanosomes (Doudoumis et al. 2013; Wang et al. 2009). Furthermore, trypanosome infections affect the behavior of tsetse flies (Bursell 1981; Dale and Welburn 2001; Weiss et al. 2013). Infected flies have been observed to feed more frequently, possibly due to increased nutrient requirements or competition for nutrients with trypanosomes (Kubi et al. 2006; Nnko et al. 2017). As a result, infected flies are more likely to transmit infections to other hosts as they actively search for additional meals compared to non-infected flies.

The primary microbiota in tsetse flies consists of *Wiggleworthia*, *Sodalis*, and *Wolbachia* (Snyder and Rio 2013). However, their strict dependence on vertebrate blood limits their acquisition of other microbes compared to mosquitoes, which have multiple food sources and, consequently, a more diverse microbiota (O'Neill et al. 1993; Osei-Poku et al. 2012). It is important to note that both male and female tsetse flies can transmit trypanosomes, making them potential targets for elimination and eradication efforts.

Understanding the roles of symbiotic microbiota in tsetse flies is crucial for combating African trypanosomiasis and improving area-wide integrated pest management (IPM) with SIT component. For example, Sodalis glossinidius, a commensal enteric microbe found in all tsetse species' organs, has been extensively studied in various paratransgenesis studies (Balmand et al. 2013; Kame-Ngasse et al. 2018; Kendra et al. 2020). However, its exact role in the host remains inconclusive. Some studies suggest that Sodalis enhances the fly's capacity to transmit trypanosomes by promoting their multiplication in the midgut and salivary glands through immune system interference and the extent of this effect can vary between species as well as genotype (Channumsin et al. 2018; Demirbas-Uzel et al. 2021; Geiger et al. 2007). Conversely, other studies indicate that Sodalisinfected flies are resistant to trypanosome infections (Dennis et al. 2014), Additionally, Sodalis has been found to influence the lifespan of tsetse flies and some reports have not defined a specific function for Sodalis (Geiger et al. 2005; Trappeniers et al. 2019).

Furthermore, the infection of salivary gland hypertrophy virus (SGHV), a DNA virus, is of particular concern, especially in tsetse mass-rearing facilities, where it reduces lifespan and induces sterility in both male and female flies, limiting colony expansion (Lietze et al. 2011). SGHV significantly affects *G. pallidipes* more than other tsetse species where the infected flies exhibit hypertrophied salivary glands, which impair the quality of saliva produced and increase trypanosomes colonization in the salivary gland and well as their transmission by the host. The infection has caused colony collapses in Ethiopia before control strategies were implemented (Abd-Alla et al. 2007, 2016). SGHV infections continue to hinder the establishment and expansion of *G. pallidipes* colonies, especially when multiple tsetse species are kept together.

SGHV has been reported in several tsetse species in infested African countries (Kariithi et al. 2013; Ouedraogo

et al. 2018), and in Tanzania, where various *Glossina* species occur (Daffa et al. 2013). SGHV infections have been detected in *G. Pallidipes, G. swynnertoni, G. fuscipes fuscipes,* and *G. morsitans morsitans.* Interestingly, the prevalence of SGHV infections was found to be higher in flies from the coastal area compared to flies from the mainland (Malele et al. 2013).

Screening wild tsetse flies before colonization is a crucial step to establish healthy and productive colonies while minimizing the risk of introducing trypanosomes and other harmful microbiota to the insectary. *G. pallidipes*, which is one of the seven tsetse fly species infesting Tanzania, is widely distributed across ecological zones and is a potential vector of trypanosomes. The species also plays a significant role in trypanosomes transmission in approximately 15 countries in eastern, southern, and central Africa (Moloo 1993; Ouma et al. 2011).

While successfully mass-rearing of *G. pallidipes* in insectaries has been challenge in Tanzania, the information available from laboratory and field studies, including its interactions with other microorganisms also is limited. The availability of that information has the potential to enhance strategies aimed at eliminating this species. Therefore, this study aimed to determine the prevalence of *Sodalis*, SGHV, and trypanosomes in wild *G. pallidipes* and explore the possible associations among them.

The results of this study contribute to the existing knowledge on the prevalence of SGHV, *Sodalis* and trypanosomes in *G. pallidipes* in Tanzania. Additionally, the implications of these findings for SIT targeting *G. pallidipes* are discussed.

Material and methods

Tsetse collection

A cross-sectional study was conducted in the Makao wildlife management area (WMA) of Meatu district, Tanzania in July 2019, which coincided with the mid dry season. The study site is located geographically between coordinates 3.4979°S and 34.3310°E. Tsetse flies were collected using POC (propyl phenol + octanol + p-cresol) and Acetone-baited NGU (Brightwell et al. 1987) and NZI (Mihok 2007) traps placed in four designated blocks (Lorugumi, Maboksini, Mbuyuni, and Shushuni) at the interface of the Makao WMA. These blocks corresponded to Makao, Iramba ndogo, Mwangudo, and Sungu villages, respectively (Fig. 1). The blocks were approximately 5-10 km apart from each other. Six traps of each type were deployed at 12 specific sites within a block, and the trapping was conducted for three consecutive days with harvests performed every 24 h. Tsetse flies collected were then identified to the species level using the Food and Agriculture Organization (FAO) training manual (FAO



Fig. 1 Map of Meatu district showing the study sites

1982). A total of 240 non-teneral flies were dissected, and their midgut and mouthparts were preserved in 95% ethanol, transported to the Vector and Vector-Borne Diseases (VVBD) Molecular Laboratory, and stored at -20 °C until further analysis.

Molecular identification of trypanosomes, *Sodalis* and SGHV infections

DNA extraction

DNA extraction was performed on the preserved samples prior to conducting Polymerase Chain Reactions (PCR). The molecular analysis aimed at identification of parasites (trypanosomes), symbionts (*Sodalis*) and the infection (SGHV) The preservation alcohol was removed first, and the samples were air-dried overnight at a temperature of 25 °C. The QIAGEN[®] DNeasy[®] Blood and Tissue Kit (Qiagen Sciences, MD, USA) was utilized for DNA extraction following the manufacturer's instructions. To ensure proper homogenization, each sample was processed using a sterile hand pestle.

As spectrometry was not available, the quality of the extracted DNA was assessed using the 2% agarose gel method described in the study conducted by Zayats et al. (2009). This involved the use of a known and labeled DNA ladder (Quick-Load 100 bp DNA ladder, Biolabs, New England, UK) and Ethidium bromide stain (Lot number 127H3719 Sigma Aldrich, Germany). The quality of DNA samples was visually examined on the gel, focusing on the intensity and size of the DNA fragments relative to the ladder fragments. These observations served as criteria to determine the suitability of the DNA. Out of the 240 extracted samples, a total of 146 samples exhibited satisfactory DNA quality and were considered suitable for further molecular studies. Samples that did not meet the quality criteria, either due to DNA degradation or absence of DNA, were excluded from the analysis.

Amplification and identification of target parasite, infection and symbiont

Three separate PCR assays were conducted to target specific organism. *Sodalis glossinidus* was identified using primers developed by Darby et al. (2005), while GpSGHV primers by Abd-Alla et al. (2007) were employed for SGHV identification. Trypanosomes were detected and identified by focusing on the Internal Transcribed Spacer-1 (ITS-1) region of the rDNA using primers developed by Njiru et al. (2005). To distinguish human infective *T. brucei rhodesiense* from *T. brucei sl* species previously detected in ITS1-PCR, a species-specific PCR assay for the serum resistance-associated gene (SRA) was performed, following a protocol and primers by Radwanska et al. (2002) (refer to Table 1). All PCR conditions followed the methodology outlined in Malulu et al. (2019).

Data analysis

A two-way analysis of variance (ANOVA) was employed to compare the number of catches between sexes and blocks using the Kruskal–Wallis test. The normal distribution of the data was assessed using the Kolmogorov–Smirnov test. Apparent density, a measurement used to estimate the tsetse population, was calculated following the methods described in Nthiwa et al. (2015) and Eyasu et al. (2021).

To examine the differences in the prevalence of trypanosome, *Sodalis*, and SGHV infections, as well as co-existence of SGHV infection and *Sodalis* between blocks and sexes, a Chi-square test was performed.

Furthermore, a multiple logistic regression analysis was conducted to establish the association between the prevalence of trypanosome infections, SGHV and *Sodalis*, as well as co-existence of SGHV and *Sodalis*. The strength of the association was Table 1 Primers used in the detection of SGHV, Sodalis and trypanosomes

Primer name	Primer sequence	Annealing Temperature	Reference
ITSCF	5'-CGGAAGTTCACCGATATTG-3'	58 °C	Njiru et al. (2005)
ITSBR	5'-TGCTGCGTTCTTCAACGAA-3'		
SRA	5'-ATAGTGACAAGATGCGTACTCAACGC-3,	60 °C	Radwanska et al.
	5'-AATGTGTTCGAGTACTTCGGTCACGCT-3'		(2002)
SODF	5'-TGAAGTTGGGAATGTCG-3'	55 °C	Darby et al. (2005)
SODR	5'-AGTTGTAGCACAGCGTGTA-3'		
GpSGHV1F	5'-GCTTCAGCATATTATTCCGAACATAC-3'	55 °C	Abd-Alla et al. (2007)
GpSGHV1R	5'-GATCCTGCTCGCGTAAACCA-3'		
GpSGHV2F	5'-CTTGTCAGCGCCACGTACAT-3'		
GpSGHV2R	5'-GCATTCACAGCATCCCAATTTT-3'		

measured using Odds Ratio (OR), an OR value of less or equal to1 indicated no association, an OR greater than 1 suggested association (Szumilas 2010). Additionally, correlation analyses were also performed to investigate the degree of interdependence between SGHV, Sodalis, and their interaction with trypanosome infections.

All statistical tests were conducted using Version 20 of the MedCalc[®] statistical software (MedCalc[®] 2021). A significance level of p < 0.05 and a 95% confidence interval were used for all tests.

Results

Apparent tsetse fly density and prevalence of Trypanosomes

A total of 1,344 G. pallidipes tsetse flies were captured during the study period, resulting in an overall apparent density



Fig. 2 Comparison of tsetse catches between sex and blocks (The bars in the graphs are standard errors at 95% confidence interval)

of 9 flies per trap per day (FTD). The comparison of tsetse catches among different traps did not show a significant difference. However, there was a significant difference in the number of female flies compared to males (p < 0.001), with a higher abundance of females. Additionally, the number of catches varied significantly between blocks (p < 0.001). The Maboksini block had the highest number of catches, followed by the Mbuyuni block, while the Lorugumi and Shushuni blocks had the lowest catches (refer to Fig. 2 for a graphical representation). For detailed analysis results on the infection status of trypanosome, Sodalis, and SGHV in G. pallidipes tsetse flies within the three blocks, please refer to Supplement Information (SI) Table 1.

The overall prevalence of trypanosome infection in G. *pallidipes* was 12.3% (n = 146) as shown in Table 2. The prevalence differed significantly between sexes and blocks (p < 0.05). Female flies exhibited a higher infection rate compared to males (p < 0.05). Among the different blocks, the Lorugumi block had the highest prevalence of trypanosome infection, while in Mbuyuni and Shushuni only one trypanosome species was detected in each block.

The majority of infected tsetse flies (89%) were found to be infected by a single species of trypanosome, while the remaining flies (11%) were infected by two different

 Table 2
 Prevalence of Trypanosomes in Glossina pallidipes

Factor	Variable	Trypanosome prevalence (%)
Block	Lorugumi	11/27 (40.7)
	Maboksini	5/51(9.8)*
	Mbuyuni	1/38 (2.6)
	Shushuni	1/30 (3.3)
Total		18/146 (12.3)

*Values indicate by an asterisk in superscript indicate the block with the only male fly which was trypanosome positive



trypanosome species. Among the identified trypanosome species, *Trypanosoma brucei brucei* accounted for 38% of the infections, followed by *T. congolense* Kilifi at 22.2%, and *T. vivax* at 16.7%. The least common infections were caused by *T. simiae* Tsavo and *T. congolense* Savannah.

Significant differences in the distribution of trypanosome species were also observed among the four blocks. For instance, the Lorugumi block accounted for 61% of all identified trypanosome species, as well as the majority of single infections (Fig. 3). Within this block, 75% of all *T. congolense* Kilifi infections (4) and 33.3% of all *T. vivax* infections (3) were found. On the other hand, the Maboksini block exhibited a higher diversity of trypanosome species and hosted all of the double infections. In Shushuni block only *T. b. brucei* was detected whereas in Mbuyuni only *T. vivax* was identified.

Prevalence of Sodalis and SGHV infections in G. pallidipes

The overall prevalence of *Sodalis* and SGHV and their interaction is shown in Table 3.

According to the results obtained from *Sodalis* and viral PCR analyses, it was found that 68.5% of the flies examined were infected with either *Sodalis*, SGHV, or a combination of both. Among the infections, the prevalence of *S. glossinidius* (*Sodalis*) only, accounted for 51.4% of the cases (Table 3). The occurrence of infections varied significantly across different blocks and between the sexes (p < 0.0001), with the highest prevalence observed in the Mbuyuni and Shushuni blocks. In the Maboksini block, no instances of single *Sodalis* were detected; instead, they co-existed with SGHV.

Table 3Prevalence of Sodalis,SGHV, and co-existence ofSGHV and Sodalis in Glossinapallidipes

Tsetse Species	Block	Sodalis only prevalence (%)	SGHV only prevalence (%)	Sodalis and SGHV prevalence (%)
G. pallidipes	Lorugumi	16/27 (59.3)	2/27 (7.5)	9/27 (33.3)
	Maboksini	0/51 (00)	9/51 (17.6)	2/51 (3.9)
	Mbuyuni	30/38 (78.9)	0/30 (0.0)	2/30 (6.7)
	Shushuni	29/30 (96.7)	0/30 (0.0)	1/30 (3.3)
Overall	4	75/146 (51.4) ^b	11/146 (7.5) ^b	14/146 (9.6) ^a

^aValues in superscript indicated by the same lower-case letter do not differ significantly

 $^{\rm b}Values$ in superscript indicated by the different lower-case letter signifies significant difference among blocks at the 5% level

The prevalence of single SGHV infections was 7.5% and limited to the Maboksini (7.5%) and Lorugumi (17.6%) blocks. There was no statistically significant difference in viral infection between females and males (p=0.9097). However, mixed infections involving both SGHV and *Sodalis* were detected in 9.6% of the flies across all blocks, with the highest occurrence observed in the Lorugumi block (33.3%).

Associations and correlation of *Sodalis* and SGHV with trypanosome occurrence

From the Tables 4 it is important to highlight that among the flies infected with trypanosomes, 67% of them also carried either SGHV, *Sodalis*, or both, while the remaining 33% had none of these infections. Specifically, single infections of SGHV were only observed in the presence of *T. simiae* Tsavo. The flies that had *Sodalis* were more commonly associated with *T. brucei brucei* (4 cases) and *T. vivax* (1 case). On the other hand, co-existence of *Sodalis* and SGHV were found to co-occur with the single infections of *T. brucei brucei*, *T. congolense* Kilifi, and *T. vivax*.

According to Table 4, there was no significant association found between flies that harbored *Sodalis* only (*Sodalis*+) and the acquisition of trypanosomes (p>0.05). Similarly, there was no significant association observed with the occurrence of trypanosomes and SGHV (SGHV+) only (p>0.05, r=-0.02811).

However, a significant association was found between the occurrence of trypanosomes and the co-existence of *Sodalis* and SGHV infections in the flies (p=0.012). Flies that had both *Sodalis* and SGHV were five times more likely to develop trypanosomes compared to other flies. This suggests that an increase in the co- existence of *Sodalis* and SGHV leads to a higher prevalence of trypanosome infections (r=0.3024).

Regarding the individual Chi-squared tests for independence conducted for each block, no significant deviations from independence were observed, except for the Maboksini block in relation to the association between SGHV and *Sodalis*, and the Lorugumi block in relation to the association between *Trypanosoma* infection and *Sodalis*. For more detailed information on these associations, please refer to the Supplementary Information (SI), specifically Table 1.

 Table 4
 Associations of Sodalis, SGHV, and co-existence of Sodalis

 and SGHV with trypanosome infection in G. pallidipes

Variable	Percentage	Odds ratio (OR)	95% CI	<i>p</i> -value
Sodalis ⁺	27.8%	0.45	0.14 – 1.49	0.1955
SGHV ⁺	33.3%	0.58	0.06 - 5.02	0.6267
Co-existence of Sodalis ⁺ and SGHV ⁺	5.5%	5.31	1.44 – 19.62	0.0122

Discussion

The study findings indicate that G. pallidipes was the dominant species over G. swynnertoni, with an average of 9 flies per trap per day (FTD). The highest FTD was observed in Maboksini (22 FTD) and Mbuyuni (12 FTD), while the lowest counts were in Lorugumi (3 FTD) and Shushuni (1 FTD). These results differ from previous studies in Serengeti National Park (SENAPA) (Auty et al. 2012) and the Serengeti ecosystem, where G. swynnertoni was reported as the most abundant species. The difference can be attributed to the use of different sampling devices and odors. Recent studies using Epsilon and/or F3 traps have shown also the dominance of G. swynnertoni (Ngonyoka et al. 2017; Nnko et al. 2017; Salekwa et al. 2014), whereas our study's findings align with studies conducted in southern Tanzania, which revealed a dominance of G. pallidipes (Luziga et al. 2017). Additionally, the study indicates that both NZI and NGU traps are equally effective in capturing G. pallidipes, suggesting that either trap can be used for sampling and control purposes.

The study reported an overall trypanosome prevalence of 12.3%. It identified a total of 7 trypanosome species and subspecies, with single infections (89%) being more common than double infections (11%) in G. pallidipes. The prevalence recorded in this study was higher than in previous studies conducted in the same areas and in East Africa (Dieng et al. 2022; Makhulu et al. 2021; Malulu et al. 2019; Nthiwa et al. 2015). The diverse range of trypanosomes found in G. pallidipes highlights the potential risk that livestock in the respective villages are exposed to. Furthermore, no human infective trypanosomes were identified in our study, in contrast to other studies conducted within SENAPA (Auty et al. 2012). However, these results are consistent with studies conducted in wildlife-human-interface areas (Ngonyoka et al. 2017; Nnko et al. 2017; Salekwa et al. 2014), suggesting that although the vector persists at the interface, the risk of acquiring human infective trypanosomes is relatively low. This may be attributed to the use of trypanocides in cattle treatment where the human infective trypanosomes are also susceptible (Kibona et al. 2006; Matovu et al. 1997) and the lower tsetse fly density at the wild interface due to farmers' application of insecticides (Lord et al. 2020).

In Maboksini, where the highest vector catches were observed, single infections of *Sodalis* were not identified, except for a few numbers of co-exitances of *Sodalis* and SGHV. Conversely, the number of trypanosome infections identified in Maboksini were also lower compared to Lorugumi, which had the fewest flies. This difference can be attributed to the high use of trypanocides in Maboksini which is also common in agropastoral system (Ngumbi and Silayo 2017). Moreover, the misuse of antibiotics in diseases management may have negatively affected *Sodalis* density in tsetse flies feeding on antibiotic treated cattle. Maboksini was an asylum providing pastures to cattle during the drought season thus abundant livestock migration were seen hence diverse *Trypanosoma* species observed. Livestock movement in tsetse infested areas can serve as vehicle in trypanosomes circulation and distribution (Selby et al. 2013). On the other hand, Lorugumi, located at the meeting point of Ngorongoro Conservation Area, Maswa Game Reserve, and SENAPA experienced extensive interaction with diverse wildlife animals and livestock thus the tsetse fly in this area had higher chances to acquire microbiome including infective trypanosome and symbionts.

The study found that the prevalence of *Sodalis* was lower than in a study by Dieng et al. (2022) but higher than in a study by Dennis et al. (2014). No association was found between harboring *Sodalis* and trypanosome infection in *G. pallidipes*, consistent with the observations of (Dennis et al. 2014) and a study conducted in Cameroon on *G. tachinoides* and *G. palpalis* (Kame-Ngasse et al. 2018). However, other studies have shown conflicting results regarding the relationship between *Sodalis* and trypanosomes (Channumsin et al. 2018; Wamwiri et al. 2014; Wongserepipatana 2016).

The prevalence of SGHV in *G. pallidipes* in this study was similar to mainland flies reported earlier (Malele et al. 2013) but lower than in coastal samples (Kariithi et al. 2013). The prevalence is also lower than of other species (Mbewe et al. 2015). Co-existence of SGHV and *Sodalis* was found to reduce *G. pallidipes'* resistance to trypanosome infections, although further investigations including laboratory studies are needed to confirm this observation, the inter-community dynamics among microbiota and their interaction may also influence different outcomes in the host, sex, and species (Wang et al. 2013) SGHV infection has been reported to alter saliva composition, leading to enhanced pathogen colonization in the salivary gland and increased feeding frequencies, affecting the vector's capacity to transmit diseases (Telleria et al. 2014).

A negative correlation was observed between *Sodalis* and SGHV. Areas with higher SGHV infections recorded no *Sodalis* infections, and vice versa. This finding is consistent with a study on *G. pallidipes* and other flies in the *morsitans* group reported by Demirbas-Uzel et al. (2021). However, In that study contrasting results were observed in the palpalis group, suggesting that SGHV may have different effects among savannah and the riverine tsetse subgroups.

Conclusions

This study provides insights into tsetse fly populations and trypanosome infections in the study area. Trypanosome infections were prevalent in *G. pallidipes*, posing a risk to

livestock. While no human infective trypanosomes were found, continuous monitoring is necessary at the wildlifehuman interface. The association between *Sodalis* and trypanosomes was not significant, but further research is needed for genetic characterization. SGHV prevalence in *G. pallidipes* was consistent with previous reports, and its co- existence with *Sodalis* affected the vector's susceptibility to trypanosomes. Overall, this study enhances our understanding and highlights the importance of surveillance and control strategies for trypanosome infections in both livestock and humans.

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Author contributions IIM, DJM and HSN, Conceived and designed the study. DJM, IIM, HSN, PL and DE executed the experiments. DJM and IWT analyzed the data and DJM, IIM, IT and AA wrote the paper.

Data availability The data that support the findings of this study are available on request from the corresponding author DJM.

Declarations

Competing interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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