Prevalence of trypanosomes and selected symbionts in tsetse species of eastern Zambia

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Abstract

Insect symbionts have attracted attention for their potential use as anti-parasitic gene products in arthropod disease vectors. While tsetse species of the Luangwa valley have been extensively studied, less is known about the prevalence of symbionts and their interactions with the trypanosome parasite. Polymerase chain reaction was used to investigate the presence of Wolbachia and Sodalis bacteria, in tsetse flies infected with trypanosomes (Trypanosoma vivax, Trypanosoma congolense and Trypanosoma brucei). Out of 278 captured tsetse flies in eastern Zambia, 95.3% (n = 265, 95% CI = 92.8–97.8) carried endosymbionts: Wolbachia (79.1%, 95% CI = 73.9–83.8) and Sodalis (86.3%, 95% CI = 81.7–90.1). Overall, trypanosome prevalence was 25.5% (n = 71, 95% CI = 20.4–30.7), 10.8% (n = 30, 95% CI = 7.1–14.4) for T. brucei, 1.4% (n = 4, 95% CI = 0.4–3.6) for both T. congolense and T. vivax, and 0.7% (n = 2, 95% CI = 0.1–2.6) for T. b. rhodesiense. Out of 240 tsetse flies that were infected with Sodalis, trypanosome infection was reported in 40 tsetse flies (16.7%, 95% CI = 12.0–21.4) while 37 (16.8%, 95% CI = 11.9–21.8) of the 220 Wolbachia infected tsetse flies were infected with trypanosomes. There was 1.3 times likelihood of T. brucei infection to be present when Wolbachia was present and 1.7 likelihood of T. brucei infection when Sodalis was present. Overall findings suggest absence of correlation between the presence of tsetse endosymbionts and tsetse with trypanosome infection. Lastly, the presence of pathogenic trypanosomes in tsetse species examined provided insights into the risk communities face, and the importance of African trypanosomiasis in the area.

Introduction

African trypanosomiasis, caused by protozoa belonging to the genus Trypanosoma, is a vector-borne disease endemic in sub-Saharan Africa. African trypanosomes are transmitted to the mammalian hosts by the bite of an infected tsetse fly (Diptera: Glossinidae) causing a fatal disease commonly known as Nagana in cattle and sleeping sickness in humans (WHO, 2017; Franco et al., 2020; Franco et al., 2022). Trypanosoma congolense is the major cause of African animal trypanosomiasis (AAT) in eastern and southern Africa whilst Trypanosoma vivax (together with Trypanosoma congolense) is a more important cause of AAT in cattle in West Africa (Cox et al., 2010; Lohaasinnarong et al., 2015; Mulenga et al., 2021). The 2 human-infective trypanosome sub-species are Trypanosoma brucei gambiense (found in west and central Africa), which accounts for over 98% of reported cases of sleeping sickness, and Trypanosoma brucei rhodesiense (found in eastern and southern parts of Africa, including Zambia), which only accounts for less than 2% of reported cases (Nakamura et al., 2019; Franco et al., 2020).

Tsetse flies host the following 3 endogenous symbionts: Wigglesworthia glossinidius, Sodalis glossinidius and Wolbachia (Wamiri et al., 2013; Makhulu et al., 2021). Wigglesworthia, found in all tsetse flies, provides nutritional and immunological benefits to its tsetse host. In the absence of this bacteria, intrauterine larval development is stunted, and progeny aborted (Weiss and Aksoy, 2011). Wigglesworthia’s contracted genome, encodes an unusually high number of putative vitamin biosynthesis pathways, which support the theory that Wigglesworthia supplements its tsetse host with nutritious metabolites that are naturally present in low titres in vertebrate blood (Wang et al., 2009; Rio et al., 2012). Sodalis on the other hand can be found both intra- and extra-cellular in various tissues of tsetse flies, including midgut, body fat, milk gland, salivary glands and haemocoeal (Dououdumis et al., 2017). Sodalis contains features associated with pathogenic lifestyles, including secretion systems, which function during the tsetse’s juvenile developmental stages (Dennis et al., 2014). Sodalis can be cultured in cell free medium, and, unlike Wigglesworthia, it is usually absent in several natural tsetse populations. Lastly, Wolbachia is a wide-spread bacteria endosymbiont infecting approximately 70% of surveyed insects. It manipulates the reproductive biology of its host mechanisms, which include cytoplasmic incompatibility (CI), male killing, feminization and parthenogenesis (Wamiri et al., 2013).

Symbiotic interactions are widespread in insects (as well as animals and plants) and may provide an avenue for disease control. The use of biological methods for the control of
vector-transmitted diseases is becoming popular globally (Ricci et al., 2012; Utarini et al., 2021). Symbionts influence several aspects of the tsetse's physiology, including reproduction, nutrition and vector competence. Several studies have suggested the involvement of insect microbiota in the ability of insect disease vectors to transmit pathogens (Geiger et al., 2007; Ricci et al., 2012; Weiss et al., 2013; Hamidou Soumana et al., 2014; Makhulu et al., 2021) thus providing hope in the potential use of symbionts to control African trypanosomiasis (Medlock et al., 2013). The presence of tsetse microbiota in Zambia’s tsetse flies has been described in studies conducted by Mbewe et al. (2015) and Dennis et al. (2014) on wild tsetse flies. While the earlier study observed significant association between present endosymbiont and trypanosome infection, the later study found it difficult to establish if some tsetse microbiota could play a role in the susceptibility of tsetse flies to trypanosomiasis infection. Little is known about the presence of symbionts in tsetse species found along the Luangwa tsetse belt of the eastern province of Zambia and the role that tsetse endosymbionts may play in the transmission and control of trypanosomiasis. Thus, the potential use of endosymbionts in trypanosomiasis control seems attractive because trypanocide-based management of trypanosomiasis infected tsetse flies has also proven to be costly and not sustainable. Furthermore, increasing vector-transmitted diseases. For expected values under 5, Fisher’s exact test was used. Known positive controls of T. congoense, T. vivax, T. b. rhodesiense and T. brucei and a negative control were included in each reaction. All samples that were positive for T. brucei were subjected to a multiple PCR using a serum resistance-associated antigen (SRA) targeting primer for the detection of T. b. rhodesiense (Welburn et al., 2001; Radwanska et al., 2002; Gaithuma et al., 2019). ITS-PCR products were separated by electrophoresis (95 V for 60 min) in a 2% (w/v) agarose gel containing ethidium bromide. The separated products were then visualized under ultraviolet light in a transilluminator. Known positive controls of T. congoense, T. vivax, T. b. rhodesiense and T. brucei and a negative control were included in each reaction. All samples that were positive for T. brucei were subjected to a multiple PCR using a serum resistance-associated antigen (SRA) targeting primer for the detection of T. b. rhodesiense (Welburn et al., 2001; Radwanska et al., 2002; Gaithuma et al., 2019).

Statistical analysis

The prevalence data of trypanosome and symbiont infection from captured tsetse flies were summarized as frequencies and percentages and analysed using descriptive statistics in Epi-info 7.2. Odds ratios were used as measures of association. A chi-square test was used to determine statistical differences between proportions. For expected values under 5, Fisher’s exact test was used. Statistical significance was acceptable at P < 0.05. Pearson’s correlation test was used to see if the presence of symbionts correlated Materials and methods

Study area and sample collection

Polymerase chain reaction (PCR) was used in a survey of tsetse symbionts and trypanosomes in tsetse species of eastern Zambia. Taking into consideration tsetse characteristics, Epsilon endosymbionts and trypanosomes in tsetse species of eastern Zambia. Taking into consideration tsetse characteristics, Endosymbiont s of trypanosome infected tsetse flies. In areas where fly density was low, flies trapped within a moving vehicle in the trapping site was used as a supplementary method to maximize catches. Deployment of traps was determined by the availability of suitable environments to maximize tsetse catches. Each trapping site was given a unique identifier and global positioning system (GPS) coordinates recorded and maintained for cross-referencing purposes. Milking of traps was done 24 h after deployment.

Sample preparation and storage

Tsetse samples collected were stored as whole flies in well-labelled bottles containing ethanol. Each bottle contained all tsetse samples captured from one trapping site. Tsetse flies caught from supplementary techniques (e.g., moving vehicle) were stored together with samples captured from the nearest possible trapping site. Prior to storage, identification data were recorded (date of collection, location, numbers captured, sex and species). During sample preparation, captured flies were removed from ethanol storage, blotted with tissue paper towel, and left to air dry overnight at room temperature. Unique identifiers given during sample collection were maintained.

Laboratory analysis

Total genomic deoxyribonucleic acid (DNA) was extracted from individual flies after removing wings and legs. Manufacturer’s instructions on DNA extraction kits (QIAamp® DNA mini kit) were followed during the extraction process. Extracted DNA was stored in 1.5 mL tubes, labelled with unique trapping numbers related to where they were trapped. The eluted DNA was stored at 4°C for use within 12 h and at −20°C for use after 12 h.

The presence of symbionts from the extracted DNA was determined using a symbiont species-specific PCR amplification assay as described by Pais et al. (2008). Four nanograms of the extracted DNA template was used for each PCR. For identification of Sodalis, HemiF (ATGGGAAAACAAAAACATTAGCCCA) and HemR (TCAAGTGACAAAACAGATAAATC) primers (Pais et al., 2008) were used to amplify the 650-bp fragment of the haemolysin gene (accession no. AP008232). The presence of Wolbachia was detected by the amplification of a 610-bp fragment of the wsp gene with primers 81F (TGGTTCAATTAAGGTAGAAGAAAC) and 691R (AAAAATTAACACGTACTCCA) (Pais et al., 2008). For DNA quality control, the G. morsitans subsp. morsitans tubulin gene (accession no. DQ377071) were amplified with primers GmmTubF (TAGTTCTCTTCAACACTTGCTTT) and GmmTubR (TGGTTGACACTTGTCTGGTG) (Pais et al., 2008). Bacteria-specific PCR amplification conditions consisted of initial denaturation at 94°C for 2 min, followed by 30 cycles of 94°C for 30 s, 54°C for 40 s and 72°C for 1 min with a final elongation at 72°C for 7 min. For gmmtub amplification, an annealing temperature of 60°C was used. The amplification products were analysed by agarose gel electrophoresis using ethidium bromide and visualized using a transilluminator (Pais et al., 2008).

ITS-PCR was undertaken in 25 μL reaction mixtures containing primers AITS-F: CGGAAGGTCACCGATATTGC and AITS-R: AGGAGAGCTACGATCCT (Gaithuma et al., 2019), One Taq 2 @ master mix (New England BioLabs, Ipswich, MA, USA), nuclease-free water and 5 μL of extracted DNA sample. For the detection of T. b. rhodesiense, SRA F (5′-ATAGTGACAAAGATGCCTACTCAACGGC-3′) and SRA R (5′-AAATTGTTGATGTGATCTCGTGTCGTCGTC-3′) (Radwanska et al., 2002) were used (procured from Inqaba Biotec, Pretoria, South Africa). Thermocycler amplification conditions were at 94°C for 5 min, followed by 40 cycles of 94°C for 40 s, 58°C for 40 s, 72°C for 90 min and 72°C for 5 min. ITS-PCR targets the internal transcribed spacer 1 of the ribosomal RNA (100–200 copies per genome), producing different sized products for different trypanosome species (Desquesnes et al., 2001; Njiru et al., 2005; Gaithuma et al., 2019). ITS-PCR products were separated by electrophoresis (95 V for 60 min) in a 2% (w/v) agarose gel containing ethidium bromide. The separated products were then visualized under ultraviolet light in a transilluminator. Known positive controls of T. congoense, T. vivax, T. b. rhodesiense and T. brucei and a negative control were included in each reaction. All samples that were positive for T. brucei were subjected to a multiple PCR using a serum resistance-associated antigen (SRA) targeting primer for the detection of T. b. rhodesiense (Welburn et al., 2001; Radwanska et al., 2002; Gaithuma et al., 2019).

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with the presence of trypanosomes. Scores were used to determine the degree of correlation present. The scale of correlation coefficients were classified as follows: negative values (negative association), positive values (positive association), no association (0.00), very low (0.00–0.19), low (0.20–0.39), moderate (0.40–0.69), high (0.70–0.89) and very high (0.90) (Schober et al., 2018).

Results

The combined prevalence for Sodalis and Wolbachia in captured tsetse flies was 95.3% (n = 278, 95% CI = 92.8–97.8) while the overall trypanosome prevalence in captured tsetse flies was 25.5% (n = 278, 95% CI = 20.4–30.7). Trypanosome prevalence was 10.8% (n = 30, 95% CI = 7.1–14.4) for T. brucei, 1.4% (n = 4, 95% CI = 0.0–2.8) for both T. congolense and T. vivax and 0.7% (n = 2, 95% CI = 0.0–1.7) for T. rhodesiense.

Out of 278 tsetse flies that were captured for the study, a total of 237 (85.3%) flies belonged to the group of Glossina pallidipes while 41 (14.8%) were G. m. morsitans. Total symbiont infections in G. pallidipes was 94.9% (n = 225, 95% CI = 92.2–97.7) while in G. m. morsitans it was 97.6% (n = 40, 95% CI = 92.8–102.3), trypanosome infections in G. pallidipes was 26.6% (n = 63, 95% CI = 21.0–32.2) while in G. m. morsitans it was 19.5% (n = 8, 95% CI = 7.4–31.6). No significant difference was observed in both symbiont (P = 0.46) and trypanosome (P = 0.34) infections in the 2 tsetse species sampled. The prevalence of symbionts and trypanosomes in the 2 tsetse species detected by PCR is summarized in Table 1.

Table 1. Prevalence (%) of symbionts and trypanosomes in tsetse species captured in the Luangwa valley, eastern Zambia

| Tsetse species | Sodalis | Wolbachia | T. brucei | T. b. brucei | T. vivax | T. congolense | T. b. rhodesiense | Mixed infections |
|---------------|---------|-----------|-----------|-------------|----------|--------------|-----------------|-----------------|
| G. m. morsitans | 85.4% (74.6–96.2) | 80.5% (68.4–92.6) | 19.5% (7.4–31.6) | 19.5% (7.4–31.6) | 0 | 0 | 0 | 0 |
| G. pallidipes | 86.5% (82.2–90.9) | 78.9% (73.7–84.1) | 12.7% (8.4–16.9) | 8.2% (4.6–11.5) | 1.7% (0.1–3.3) | 1.7% (0.1–3.3) | 0.8% (0–3.2) | 1.7% (0.1–3.3) |

Table 2. Symbiont and trypanosome infection in relation to the sex of caught tsetse flies in the Luangwa valley, eastern Zambia

|          | Sodalis | Wolbachia | T. brucei | T. vivax | T. congolense | T. b. rhodesiense |
|----------|---------|-----------|-----------|----------|--------------|------------------|
| Female   | 158     | 146       | 17        | 2        | 1            | 1                |
| Male     | 82      | 74        | 17        | 2        | 3            | 1                |
| Odds ratio | 1.9   | 1.3       | 2.3       | 2.1      | 6.4          | 2.1              |

Discussion

The tsetse fly has established symbiotic associations with bacteria which influence its reproduction, nutrition and vector competence. Symbiotic interactions are widespread in insects (and also animals and plants) and may provide an avenue for disease control (Riccio et al., 2012; Wamiri et al., 2013). The current study provided the prevalence of selected tsetse symbionts and trypanosomes in Glossina tsetse species from eastern Zambia. Results showed no statistical difference in the prevalence of both symbionts and trypanosomes in the 2 tsetse species (G. m. morsitans and G. pallidipes) analysed. No association was either observed between symbiont and trypanosome infection in the 2 tsetse species, suggesting that endosymbionts play no role in tsetse vector competence and reproduction in the area. These data are in agreement with those obtained by Dennis et al. (2014) but disagree with those by Farikou et al. (2010) and Mbewe et al. (2015), who established the existence of a relationship between tsetse bacteria and trypanosomes and the potential role of endosymbionts in tsetse vector competence and reproduction. However, later studies were conducted in different geographical areas with different species of tsetse flies (G. p. palpalis and G. m. centralis, respectively).

Tsetse symbions (Wolbachia and Sodalis) were detected in about 95% of the tsetse samples examined with varying prevalence within tsetse species. Both symbionts were found in relative abundance in the 2 tsetse species examined, with Sodalis prevalence slightly higher than Wolbachia. This agrees with findings from similar studies on tsetse symbions though with varying levels of infection rates which may be attributed to differences in the sensitivity of the screening methods (Doudoumis et al., 2012; Dennis et al., 2014; Doudoumis et al., 2017). The low numbers of Wolbachia have been associated with low sensitivity of the standard PCR assay (Wamiri et al., 2013), which was also used in
our laboratory analysis of tsetse samples. The presence of *Sodalis* and *Wolbachia* infection in the tsetse population sampled re-affirms the presence of tsetse bacterium in tsetse species found in Zambia and particularly the Luangwa valley (Doudoumis et al., 2012; Dennis et al., 2014; Mbewe et al., 2015).

The overall trypanosome prevalence in the captured tsetse flies (25.5%) was similar to what was found by Nakamura et al. (2021). The identification of *T. congolense, T. brucei* and *T. vivax* from tsetse samples analysed confirms the presence of AAT in the community (Mekata et al., 2008; Laohasinnarong et al., 2015; Mulenga et al., 2021; Nakamura et al., 2021). The presence of *T. b. rhodesiense* further indicated the circulation of the human-infective trypanosomes in the area, responsible for sleeping sickness and the importance of the tsetse species in trypanosomiasis transmission. Taken together, the presence of pathogenic trypanosomes in tsetse species examined provide insights to the risk of contracting sleeping sickness and AAT by the local communities and their livestock (Mekata et al., 2008; Djohan et al., 2015; Auty et al., 2016).

In agreement with Mekata et al. (2008), high infections of both symbionts and trypanosomes were reported in the *G. pallidipes* species compared to *G. m. morsitans*. However, unlike observations from the current study, Doudoumis et al. (2012) found *G. m. morsitans* to be more likely to harbour *Wolbachia* than *G. pallidipes*. On the other hand, current study findings were in concordance with findings obtained elsewhere, where *G. pallidipes* was captured with other tsetse species other than *G. morsitans* (Wamiri et al., 2013). Further, the high prevalence of female *G. pallidipes* found agree with findings by Laohasinnarong et al. (2015). Overall, both symbiont and trypanosome prevalence were, however, higher in female tsetse flies than in male tsetse flies and were associated with the host tsetse species as previously reported (Wamiri et al., 2013; Dennis et al., 2014). Such findings prompt for further research in the importance of *G. pallidipes* tsetse species with regards to host genetic diversity and vectorial capacity in areas where other tsetse species are present.

The weak relationship between tsetse symbiont prevalence and trypanosome prevalence shown in the current study does not support the synergistic role between symbiont and trypanosomiasis transmission in the surveyed area. However, the low number of tsetse flies infected with trypanosomes could explain the poor correlation observed, which suggest the need for further work on the importance of *Sodalis* in tsetse species in the Luangwa valley tsetse belt. Understanding insect–parasite–symbiont interactions is necessary in establishing opportunities for biologically based trypanosomiasis control strategies (Boulanger et al., 2002). The importance of understanding this relationship is emphasized by the urgent need for environmentally friendly methods for both tsetse and trypanosomiasis control. The high prevalence of *Wolbachia* in female flies need to be investigated further as a possible basis for environmentally sustainable tsetse population control for *Glossina* species.

**Table 3. Measures of association between trypanosome and symbiont infection in tsetse flies caught in the Luangwa valley, eastern Zambia**

| Trypanosome infection | Sodalis infection |
|-----------------------|------------------|
| **T. brucei**         |                  |
| Present               | 192              | 209 | 1.7 | 0.5–6.0 |
| Absent                | 28               | 31  |      |
| **T. congolense**     |                  |
| Present               | 216              | 237 | 0.5 | 0.0–4.6 |
| Absent                | 4                | 3   | 1    |
| **T. vivax**          |                  |
| Present               | 217              | 236 | 1.3 | 0.0–6.0 |
| Absent                | 3                | 4   | 0    |
| **T. b. rhodesiense** |                  |
| Present               | 218              | 238 | 3.0 | 0.57   |
| Absent                | 2                | 0   |      |

**Table 4. Correlations between trypanosome and symbiont infection in tsetse flies caught in the Luangwa valley, eastern Zambia**

| T. brucei | T. vivax | T. congolense | T. b. rhodesiense |
|-----------|----------|---------------|-------------------|
| Pearson's correlation | Sig. (2-tailed) | Pearson's correlation | Sig. (2-tailed) | Pearson's correlation | Sig. (2-tailed) | Pearson's correlation | Sig. (2-tailed) |
| Sodalis   | 0.05     | 0.38          | −0.01             | 0.84             | −0.04  | 0.51            | 0.03              | 0.57             |
| Wolbachia | 0.03     | 0.62          | −0.01             | 0.84             | 0.06   | 0.30            | 0.04              | 0.47             |

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**Ethical standards.** Human and animal ethical clearances were obtained from James Cook University (H7226 and A2498) and the Zambian Ethics Committee (Ref. No. 2018-Oct-001), and the research was approved by the Zambia National Health Research Authority.
References

Auty H, Morrison LJ, Torr SJ and Lord J (2016) Transmission dynamics of Rhodesian sleeping sickness at the interface of wildlife and livestock areas. Trends in Parasitology 32, 608–621.

Boulanger N, Brun R, Ekeh-Sabantier L, Kunz C and Butel P (2002) Immunopeptides in the defense reactions of Glossina morsitans to bacterial and Trypanosoma brucei brucei infections. Insect Biochemistry and Molecular Biology 32, 369–375.

Cox AP, Tosas O, Tilley A, Picozzi K, Coleman P, Hide G and Welburn SC (2010) Constraints to estimating the prevalence of trypanosomiasis infections in East African zebu cattle. Parasites & Vectors 3, 82.

Dennis JW, Durkin SM, Horsley Downie JE, Hamill LC, Anderson NE and Cox AP (2013) Evaluating paratransgenesis as a potential control strategy for African trypanosomiasis. PLoS Neglected Tropical Diseases 7, e2377.

Mbekwe NJ, Mweempwa C, Guya S and Wamwiri FN (2015) Microbiome frequency and their association with trypanosomiasis infection in male Glossina morsitans centralis of Western Zambia. Veterinary Parasitology 211, 93–98.

Medlock J, Atkins KE, Thomas DN, Aksay S and Galvani AP (2013) Prevalence and source of trypanosomiasis infections in field-captured vector flies (Glossina pallidipes) in southeastern Zambia. Journal of Veterinary Medical Science 70, 923–928.

Mulenga GM, Namangala B, Chilongo K, Mbumba C, Hayashida K, Henning L and Gummbow B (2021) Challenges in the diagnostic performance of parasitological and molecular tests in the surveillance of African trypanosomiasis in eastern Zambia. Tropical Medicine Infectious Diseases 6, 68.

Wigglesworthia glossinidius influences reproduction, digestion, and immunity processes of its host, the tsetse fly. Applied and Environmental Microbiology 74, 5965–5973.

The elimination of transmission of Trypanosoma brucei rhodesiense. The American Journal of Tropical Medicine and Hygiene 67, 684–690.

The oblique mutualist Wigglesworthia glossinidius influences reproduction, digestion, and immunity processes of its host, the tsetse fly. Applied and Environmental Microbiology 74, 5965–5973.

Symbiotic control of mosquito borne disease. Pathogens and Global Health 106, 380–385.

The oblique mutualist Wigglesworthia glossinidius influences reproduction, digestion, and immunity processes of its host, the tsetse fly. Applied and Environmental Microbiology 74, 5965–5973.

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