Imperial Journal of Interdisciplinary Research (IJIR)
Vol-2, Issue-11, 2016
ISSN: 2454-1362, http://www.onlinejournal.in

Diversity of Blood Meal Hosts in *Glossina Pallidipes* and Its Role in the Epidemiology of *Trypanosomiasis* at a Localized Area in Serengeti National Park

Hamisi S Nyingilili[1], Imna I Malele[1] & Gamba Nkwengulila[2]
[1]Tanzania Veterinary Laboratory Agency, Vector and Vector borne Disease, P.O.Box 1026, Tanga, Tanzania.
[2]College of Natural and Applied Sciences, Department of Zoology and wildlife conservation, University of Dar es salaam, Tanzania.

Abstract: *Tsetse fly is an obligatory haematophagous and depends on variety of mammalian hosts for their blood meal. While tsetse flies are in feeding unfortunately they can be infecting/ transmitting or get infected by trypanosomes. Therefore understanding the host preference could contribute towards understanding the epidemiology of Trypanosome and identifying its risk potential. The study was determined to identify the diversity of blood meals taken from vertebrate hosts in wild-caught G.pallidipes in Death Valley Serengeti Tanzania.*
Flies were collected seasonally at ten days intervals in between Sept 2012 and May 2013 using tsetse traps. Residual blood meals from the flies were preserved on FTA cards, for DNA extraction, and then screening was done with cytochrome b primer pair followed by sequencing and BLASTING to identify the mammal hosts.
Forty six blood meal sources were successfully identified out of 83 samples collected. Vertebrate species identified as hosts were African buffalo 87%; Warthog 10%; African elephant 4.35% and Giraffe 2.17%.
Among the identified hosts the most feed is African buffalo. Therefore G. pallidipes plays a role in the epidemiology of HAT since the buffalo and warthog are known to harbour T.b. rhodensience.

Key words; *Host, Transmission, Glossina pallidipes, Cytochrome b, HAT, blood - meal*

1.Background

Most of haematophagous arthropods feed on a limited range of host species, with varying degrees of frequency on individual species, when given a choice [1]. Furthermore, the haematophagous arthropods "flies" nutritional behavior could depend on the presence of specific vertebrate species [2]. Identification of the food source of arthropods allows a better understanding of the vector dynamics and transmission routes of diseases carried by vectors.

Host preference in Tsetse flies varies from place to place and is influenced by factors such as tsetse fly species involved, geographical location and animal population available in the habitat / ecosystem. Though, it is believed that tsetse flies feed on whatever suitable hosts that may be available in an opportunistic way, but when there is an abundance of choices, they select their host based on olfactory and visual cues [3]. Therefore, preference mainly applies to the particular area from where tsetse flies were collected and cannot be used to make a general conclusion on host preference for tsetse flies in a large geographical area. Staak *et al*. [4] concluded that the host preference of tsetse flies differ depending on the sampling area. Information on the source of blood meals of tsetse flies is essential in understanding the relationship between hosts and vectors, and their respective roles in the trypanosomiasis transmission cycle [5]. Furthermore, blood meal analysis provides important information relating to the epidemiology of trypanosomiasis and natural feeding habits of different species of *Glossina*.

The specie of *G.pallidipes* is found in almost all parts of East Africa and known to live in a variety of habitats [6]. Several mammalian hosts have been mentioned to be fed by *G.pallidipes* at Shinyanga in Tanzania [7, 9], Southern Busoga in Uganda [8] Northern East shore of lake Victoria in Uganda [10], Masai mara in Kenya [11] and at Busia and Ngurumani in Kenya [12]. However the study on hosts of *G.pallidipes* in the Death Valley has not being done for decades. Furthermore previously mentioned animals have been conferred to be reservoirs of human infective trypanosome. Therefore it is of importance to identify the host fed on by *G,pallidipes* in view of ascertaining their contribution in the epidemiology of HAT in the area.

The present study applied molecular diagnostic techniques to detect host preference of *G.pallidipes*, where mitochondrial cytochrome b was used as marker for identifying sources of tsetse fly blood meals [13]. This was followed by

sequencing of the cytochrome b PCR product and according to the nucleotide–nucleotide basic alignment search tool (BLAST) host species identified. The approach is arguably the most viable method for determining the hosts on which tsetse flies feed. The investigation on feeding patterns of tsetse flies is essential in understanding the relationship among these strictly haematophagous vectors and their hosts. The aim of the study was therefore to determine the host preference of *G.pallidipes* using molecular marker and it implication toward the specie involvement in the epidemiology of HAT.

2. Methods

Study Area

The study was conducted in Serengeti National Park Tanzania, best known for its abundance of animals and the great wildebeest migration. The park is situated west of the rift valley and the west border is close to Lake Victoria, while northern edge borders Kenya. The valley is located 020 22" 540.3'S and 034 0 43" 120.7' E at an altitude of 1,450m. (TTRI unpubl). The valley is in the Ikoma-Seronera-Kilimafedha triangle; the centre of human activity in the Serengeti National Park and Ikoma, and where game and tsetse are abundant. Study focused on the area because of its sleeping sickness related history and occurrence of human activities in the area.

Sample collection for blood meal analysis

Glossina pallidipes were collected seasonally for ten days intervals in Sept 2012 and May 2013 in Death Valley. Flies were captured using NGU (14) and NZI tsetse traps (15), Live *G.pallidipes* was sorted into different sexes and non teneral flies were dissected under a dissecting microscope. Midguts with residual blood in the crop and/or posterior of their midgut were taken and smeared on the filter paper (Whatman® FTA- card) and left to dry at room temperature before they were kept into aluminum porches sealed with descant for preservation before laboratory analysis.

DNA extraction from FTA cards blood

A 6 mm diameter disc was punched from the centre of the dried blood in FTA cards and placed into a 1.5 ml eppendorf tube. Two hundred micro liters (200 µl) of sterile water was added into each sample and incubated at 37 ˚C for 30 minutes on the heating block. Then water was removed after incubation and 60 µl of sterile water was added in an original tube with a filter paper disc and incubated at 100 ˚C for 30 minutes [16]. The 359 bp fragment from mitochondrial cytochrome b (*cytb***)** fragment was recovered using primers CYTB 1 (5'-CCATCCAACATCTCAGCATGATGAAA-3') and CYTB 2 (5'- GCCCCTCAGAATGATATTTGTCCTCA-3') [13]. PCR was carried out in a total volume of 25 µl containing 1 µl of 10 pmol of each primer, 20 µl of pure PCR water, 2 µl of the DNA template and 1unit of read to-go bead (PuRe Taq™ Ready-To-Go™ PCR beads). PCR cycling conditions was initiated by denaturation at 94˚C for 5 minutes, followed by 35 cycles of denaturation at 94 ˚C for 30 s, annealing temperature at 55˚C for 45 sec, and primer extension at 72˚C for 30 s, with a final extension at 72˚C for 10 min

Sequencing of the PCR cytochrome b product

PCR products of positive field midgut samples, positive control (midgut of laboratory *G.pallidipes* feed on White swiss mice) and Negative control (Midgut of Teneral Laboratory *G.pallidipes* for cytochrome b were shipped to Korea for sequencing, the sequences were then used for species identification according to the nucleotide–nucleotide basic alignment search tool (BLAST) in the GenBank DNA sequence database (http://www.ncbi.nlm.nih.gov/).

Data analysis

To determine the possible differences on mitochondrial cytochrome b (*cytb***)** of animal spieces identified to be feed on by *G.pallipes* during the wet and dry season, Chi-square test were performed using Epi Info version 6.01 with a cut-off value of 0.05.

3. Results

Table 1. Blood meal samples collected during wet and dry season

Season	Male	Female	Total
Dry	19	22	41
Wet	21	21	42
Total	40	43	83

Out of Eighty three blood meal sample collected during the study. Forty one blood meal samples were obtained during the dry season 46.34% were Male and 53.66% were female. During the wet season forty two blood meal samples were collected in equal percentages between Male and female flies (Table 1). From 83 collected blood meal samples from *G.pallidipes* at Death Valley in this study only fifty seven (57) samples gave positive bands for cytochrome b gene with PCR at 359 base pair while twenty six (26) samples failed to give a signal (band).

Table 2. Host blood meal origin determined by similarity with sequences of species in the GenBank database.

Host species	Mitochondrial DNA detection rate				Gene bank access number
	Dry		Wet		
	Male	Female	Male	Female	
Giraffa camelopardalis (Giraffe)	0	0	0	1/29	HF571174
Loxodonta africana (African elephant)	0	0	0	2/29	AY768878, HQ634691,
Phacochoerus africanus (Warthog)	0	0	1/29	4/29	HQ634692, HQ634693, DQ470797 FJ85390
Syncerus caffer (African buffalo)	7/19 (36.8)	12/19 (63.3)	10/29	11/29	HQ680422, HQ680408, HQ680424, HQ680420, HQ680411, HQ680412 HQ680413, HQ680414, HQ680416, HQ680417, HQ680418 HQ680419, HQ680420, HQ684694, HQ680422, HQ680423, HQ680424 HQ680409, HQ680426, HQ680427, HQ680428, HQ680429, HQ680430

Forty eight (48) blood meal sources were successfully identified out of 57 blood meal sample which amplified cytocloma b gene in the study with no mixed blood meals observed. Four vertebrate species were identified as hosts, as listed in table 2. The most common host species was *Syncerus caffer* (African buffalo), which constituted 83.3% of the identified blood meals. The second species identified was *Phacochoerus africanus* (Warthog), which constituted 10.1% of the identified blood meals. The third observed host was *Loxodonta africana* (African elephant), which constitute 4.4% of the identified blood meals and forth identified host was *Giraffa camelopardalis* (Giraffe), which constitute 2.2% of the identified blood meal. Nine samples failed to be sequenced.

4. Discussion

Serengeti National Park has abundance of wildlife and is solaria vital for the survival of tsetse flies population. Hence tsetse flies have diversity of mammalian host for their blood meal. Four different mammalian host species were identified as the source of the blood meal for *G.pallidipes* captured from the Death Valley. The host animal species which were identified included African buffalo (*Syncerus caffer*), warthog (*Phacochoerus africanus*), Giraffe (*Giraffa camelopardalis*) and Elephant (*Laxodonta africana*).

The dominant host with the highest mammalian cytochrome b detection rate was the buffalo in both seasons. These findings suggest that the tsetse fly in this area fed more on buffalo which would therefore seem to support earlier reports on the subject in different places [17, 18, 19].

Host selection might be is due availability of hosts and not based on true preference, as tsetse are capable of adopting to new hosts in the absence of their usual ones. However in this study based on results of cytochrome b analysis, it showed high frequency of feeding on buffalo and the only host fed upon during the dry season.

Though During the dry season 22 samples were not able to be identified their blood meal original, however 19 sample were all identified with African buffalo blood original, in other end during the wet season 13 samples were not able to be identified and 29 identified sample were from African buffalo (21), African elephant (2), Giraffe (1) and warthog (5). There is significance difference on host fed by *G.pallidpes* between the wet and dry season ($p < 0.05$).

Failure to amplify cytochrome b gene by PCR in some samples was probably caused by interval of fly feeding to the time of collection; blood meal collected 96 hours after feeding of the fly will be completely degraded or will have too low concentration to be detected by PCR [15]. In the contest Steuber *et al*, [15] had showed detection rates of host DNA from blood meal of fed tsetse flies in the laboratory to be 100%, 80%, 60%, and 40% at 24, 48, 72, and 96 hours after feeding, respectively.

During the dry season buffalos were observed to be the only host feed by *G.pallidipes.* The possible reason for this observation could be buffalos were more attractive to flies than other hosts or they remained docile when flies were feeding on them. Though several other animal species were seen in the area during the dry season but none appeared to be fed on with *G.pallidipes*. However, during wet season *G. pallidipes* observed to feed on four mammalian species, namely, *Syncerus caffer* (African buffalo), *Loxodonta africana* (African elephant), *Phacochoerus africanus* (Warthog) and *Giraffa camelopardalis* (Giraffe) in that order.

These results corroborate with results from other studies in different places [18, 19, 20]. Moreover, Sasaki *et al*, [21] in Kenya have reported bushbuck and ostrich to be other hosts fed upon by *G.pallidipes.*

The difference on host fed during the wet and the dry season in this study might be caused by differences in physiological status of flies. During the wet season flies have higher longevity, which results to severe damage of their wings due to flight, mating and feeding activities thus limits their ability to locate hosts, consequently fostering extreme starvation. This make flies to be desperate to feed as it is for the young flies that have just emerged (teneral flies). Consequent disregard of host to feed on, this is probably why other host species fed upon were observed during wet season.

In other hand during the dry season tsetse flies emerge from small size pupae which have been exposed to high temperatures, thus emerged flies tend to be small in size with need of blood meal immediately. Buffalos were observed resting in the evergreen bushes, which are the lavipositing sites for tsetse flies. Therefore, the probability of teneral flies to get their first blood meal from buffaloes immediately after emergence was high. This could be a scenario as there was no other animal species observed resting quietly as buffalos in tsetse ovipositing or resting vegetation. All together it is most likely that flies couldn't fly a long distance searching for an animal to feed on when they can find one close to them. In view of the fact that long distance flying is costly energetically but also exposes them to more dangers. The first blood meal obtained has an effect on the subsequent feeds. Tsetse flies have a tendency of going back to feed on animal species which they obtained their first blood meal [22]. This could be the reason why during dry season buffalo were the only specie observed to be fed on.

G.pallidipes captured from the Death Valley in this study are shown to be feeding on African buffalo (*Syncerus caffer*), warthog (*Phacochoerus africanus*), Giraffe (*Giraffa camelopardalis*) and Elephant (*Laxodonta africana*). More over previous study on *G.swynnertoni* in the Serengeti ecosystem were also reported to feed on *Syncerus caffer* (African buffalo), *Loxodonta africana* (African elephant), *Phacochoerus africanus* (Warthog) and *Giraffa camelopardalis* (Giraffe) and *Crocuta crocuta* (Spotted hyena) [23]. Therefore the feeding hosts of these two tsetse species are almost the same species of animals. This in turn provides evidence of the involvement of all two species in the epidemiology of HAT in the area. As among of their host Warthog and Buffalo have been reported to be reservoirs of Human infective trypanosome [24, 25]

5. CONCLUSION

G. pallidipes in the Death Valley fed on *Syncerus caffer* (African buffalo), *Loxodonta africana* (African elephant), *Phacochoerus africanus* (Warthog) and *Giraffa camelopardalis* (Giraffe). The most fed host is *Syncerus caffer* (African buffalo). *G. pallidipes* plays a significant role in the epidemiology of HAT in the study area since the buffalo and warthog are known to habour *T.b. rhodensience*

Acknowledgement

We thank, Agricature Strategic Development Pan (ASDP) under Ministry of Livestock and Fisheries for fund Serengeti National park for support,. Lupaksyo Kapange, Eugene Lyaruu, Dr Idrisa Chuma, Deusdedt Malulu and Jerome Mtundu for valuable contributions to execute and complete this study.

6. References

[1].Burket- Cadena ND, Graham SP, Hassan HK, Guyer C., Eubanks MD: Blood feeding patterns of potential arbovirus vectors of the genus culex targeting ectothermic hosts. *Am J Trop Med Hyg* 2008, **79:** 809 – 15

[2]. Mukabana WR, Takken W, Knols BG: Analysis of arthropod bloodmeals using molecular genetic markers. *Trends Pa*rasitol 2002, **18**: 505–509

[3]. Willemse LP, Takken W: Odor-induced host location in tsetse flies (Diptera: Glossinidae*). J Med Entomol* 1994, **31:** 775 – 94

[4].Staak C, Kampe U, Korkowski G: Species identification of blood meals from tsetse flies (Glossinidae), Results 1979-1985. *Trop Med Parasitol* 1986, **37:** 59 – 60

[5].Tempelis CH: Host feeding patterns of mosquitoes, with a review of advances in analysis of blood meals by serology. *J Med Entomol* 1975, **11:** 635 – 653

[6]. Jordan AM: Tsetse-flies (Glossinidae). In *Medical insects and arachnids* (Ed. by R. P. Lane & R. W. Crosskey) Chapman & Hall, 1993. 332-388.

[7]. Glowsgow JP, Isherwood F, Lee-jones F, Weitz B: Factors influencing the steple food of tsetse flies. *Journal of Animal Ecology* 1958, **22**: 129 – 137

[8]. Southon HAW: Hosts of Glossina in South Busoga, Uganda. East African Trypanosomiasis Research Organization. *Annual Report*; 1961, 1963

[9].Weitz B, Glasgow JP: The natural hosts of some species of *Glossina* in East Africa, *Trans. Rec. Soc. Trop. Med. Hyg* 1956, **50** (6): 593 – 612

[10]. Moloo SK, Kutuza SB, Boreham, PFL: Studies on *Glossina pallidipes, G. fuscipes fuscipes* and *G. brevipalpis* in terms of the epidemiology and epizootiology of trypanosomiasis in south-

eastern Uganda. *Annals of Tropical Medicine and Parasitology* 1980, **74**: 219-237
[11]. FAO 1982, Tsetse *Control Training Manual, Vol.2, Ecology and Behaviour of Tsetse*. Pollock JN (Ed) Food and Agriculture Organization of the United Nations, Rome
[12].Okoth SO: *Glossina pallidipes* and host interactions: Implications of host preference on transmission risk of Rhodesian sleeping sickness in Kenya. *Trends in Applied Sciences Research* 2007, **2**(5): 386 – 394.
[13].Brightwell R, Dransfield RD, Kyorku C, Golder TK, Tarimo SA. and Mungai D 1987 A new trap for *Glossina pallidipes*. *Tropical Pest Management.* **33:** 151 – 159.
[14].Mihok S 2002 The development of a multipurpose trap (the NZI) for tsetse and other biting flies. *Bulletin of Entomological Research.* **92**: 385 – 403.
[15].Steuber S, Rady AA, Clausen PH: PCR-RFLP analysis: a promising technique for host species identification of blood meals from tsetse flies (Diptera, Glossinidae). *Parasitol Res* 2005, **97:** 247–254
[16].Boid R, Jones TW, Munro A: A simple procedure for the extraction of trypanosome DNA and host protein from dried blood meal residues of haematophagous Diptera *Vet. Parasitol* 1999, **85**: 313-317
[17].Clausen PH, Wiemann A, Patzlt R, Kakaire D, Poetzsch C, Peregrine A, Mehlitz D: Use of PCR assay for the specific and sensitive detection of *Trypanosoma* spp in naturally infected dairy cattle in peri-urban Kampala, Uganda. *Annales of New York Academic Science* 1998,. **29:** 21–31
[18].Simo G, Njiokou F, Mbida Mbida JA, Njitchouang GR, Herder S, Asonganyi T, Cuny G: Tsetse fly host preference from sleeping sickness foci in Cameroon: Epidemiological implications *Infection, Genetics and Evolution* 2007, **8**: 34-39.
[19].Konnai S, Mekata H, Odbileg R, Simuunza M, Chembensof M, Witola WH,Tembo ME, Chitambo H, Inoue N, Onuma M, Ohashi K: Detection of *Trypanosoma brucei* in field-captured tsetse flies and identification of host species fed on by the infected flies. *Vector Borne Zoonotic Dis* 2008, **8:** 565 - 573
[20].Mekata H, Konnai S., Simuunza M., Chembensofu M.,. Kano R, Witola WH., Tembo ME., Chitambo H., Inoue N.,. Onuma M . Ohashi K: Prevalence and source of trypanosome infections in field-captured vector flies (*Glossina pallidipes*) in southeastern Zambia. *Journal of Veterinary Medical Science* 2008, **70**: 923 - 928
[21].Sasaki H., Kang'ethe EK. Kaburia HF: Blood meal sources of *Glossina pallidipes* and *G. longipennis* (Diptera: Glossinidae) in Nguruman, southwest Kenya. *J Med Entomol* 1995, 32:,390 – 393
[22].Bouyer J, Ravel S, Vial L**.,** Thévenon S, Dujardin JP: DeMeeus T: Population structuring of *Glossina palpalis gambiensis* (Diptera:Glossinidae) according to land scape fragmentation in the Mouhounriver, Burkina Faso. *J.Med.Entomol* 2007, **44**: 788 –795
[23].Muturi CN, Ouma JO, Malele II, Ngure RM, Rutto JJ, Mithofer KM, Enyaru J, Masiga DK: Tracking the feeding patterns of tsetse flies (Glossina genus) by analysis of blood meal using mitochondrial cytochrome b genes. *PLoS One* 2011 **6**(2): 1-6
[24].Kaare MT, Picozzi K, Mlengeya T, Fe`vre EM, Mellau L, Mtambo MM, Cleaveland S, Welburn S: Sleeping sickness A re-emerging disease in the Serengeti? *Travel Medicine and Infectious Disease* 2007, **5:** 117–124
[25] Anderson NE, Mubanga J, Fevre EM, Picozzi K, Eisler MC, Thomas R, Welburn SC: Characterisation of the wildlife reservoir community for human and animal trypanosomiasis in the Luangwa Valley, Zambia. *PLoS Negl Trop Dis* 2011, **6:** e1211