



Vector competence of *Glossina austeni* and *Glossina brevipalpis* for *Trypanosoma congolense* in KwaZulu-Natal, South Africa

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Tsetse-transmitted trypanosomosis (nagana) has been the cause of stock losses in the recent past and still presents a major problem to livestock owners in certain areas of KwaZulu-Natal, South Africa. Over 10 000 cattle mortalities were reported in the 1990 nagana outbreak. Although information on the distribution and abundance of the tsetse flies *Glossina brevipalpis* and *Glossina austeni* in KwaZulu-Natal exists, data on their vector competence are lacking. This study aimed to determine the rate of natural *Trypanosoma congolense* infection by field-collected as well as colony-reared flies of these species. A total of 442 field-collected *G. brevipalpis* and 40 *G. austeni* flies were dissected immediately after collection to determine their infection rates, whilst 699 *G. brevipalpis* and 49 *G. austeni* flies were fed on susceptible animals in 10 and four batches, respectively, for use in xenodiagnosis experiments. Teneral colony flies were fed on infected animals and dissected 21 days post infection to confirm their infectivity testing. *Glossina austeni* harboured 8% immature and mature infections. In *G. brevipalpis*, the infection with the immature stages was lower (1%) and no mature infections were observed. Although all four batches of *G. austeni* transmitted *T. congolense* to four susceptible animals, no transmission resulted from 10 batches of *G. brevipalpis* fed on susceptible cattle. Colony-derived *G. austeni* (534) and *G. brevipalpis* (882) were fed on four bovines infected with different *T. congolense* isolates. Both *G. austeni* and *G. brevipalpis* acquired trypanosome infection from the bovines, with immature infection ranges of 20%–33% and 1%–4%, respectively. Parasites, however, only matured in *G. austeni* (average = 4%). *Glossina austeni* plays a larger role in the epidemiology of animal trypanosomosis in KwaZulu-Natal than *G. brevipalpis* and therefore more focus should be aimed at the former when control measures are implemented.

Introduction

The tsetse zone in South Africa encompasses an area of 18 000 km², representing the southernmost distributional limit of this genus in Africa (Kappmeier-Green, Potgieter & Vreysen 2007). Of the four tsetse species (Diptera: Glossinidae) historically found in South Africa, only *Glossina austeni* (savanna species) and *Glossina brevipalpis* (fusca species) still exists in the north-eastern parts of KwaZulu-Natal. The fly habitat is confined to protected nature reserves, game parks, indigenous forests and river beds (Kappmeier, Nevill & Bagnall 1998). The other two species, namely *Glossina morsitans morsitans* and *Glossina pallidipes*, which were considered to be the most efficient vectors of trypanosomes in general, had been eradicated in 1897 and 1954, respectively (Du Toit 1954). *Glossina austeni* and *G. brevipalpis* were not considered to be important vectors of trypanosomosis in South Africa (Fuller 1923).

Despite the eradication of *G. morsitans morsitans* and *G. pallidipes*, clinical cases of trypanosomosis were still diagnosed in cattle, horses and dogs between 1955 and 1989 (Bagnall 1993). In 1990, a serious outbreak of the disease in cattle, locally referred to as nagana, was reported at dip tanks in the vicinity of the Hluhluwe–Umfolozo Game Reserve and the infection was attributed to *Trypanosoma congolense* and *Trypanosoma vivax*. The treatment of cattle with trypanocidal drugs, combined with tsetse control efforts using deltamethrin 'pour-on' insecticide and odour-baited, insecticide-impregnated targets, was successful in controlling the outbreak (Bagnall 1993), but the strategy was considered unsustainable because preventing tsetse fly reinvasion could not be guaranteed (Kappmeier *et al.* 1998). The temporary nature of this approach was evidenced by the recurrence of trypanosomosis, which has reverted to the high levels seen in 1990, before these temporary control measures were instituted (Van den Bossche *et al.* 2006). The reoccurrence of outbreaks changed the previous perception that *G. austeni* and *G. brevipalpis* were of minor importance in trypanosome transmission and reaffirmed that they are responsible for the cyclical transmission of nagana (Kappmeier & Nevill 1999; Kappmeier-Green *et al.* 2007). Subsequent

tsetse surveys conducted from 1991 to 1999 revealed a relative higher prevalence and abundance of *G. brevipalpis* compared with that of *G. austeni* in the affected areas (Bagnall 1993; Kappmeier 2000). Recently, a study conducted at the Mvutshini dip tank found trypanosome infections in 61% of suspected sick cattle using the buffy coat procedure (Van den Bossche *et al.* 2006). The survey indicated that nagana was still prevalent in KwaZulu-Natal and that *T. congolense* was the dominant causative organism. Of the two tsetse species, *G. brevipalpis* was found to be more abundant and therefore believed to be responsible for causing disease in cattle around the Hluhluwe–Umfolozu Game Reserve. The importance of a species as a vector is not determined only by its abundance, but also by its vector competence; that is, its ability to become infected and transmit pathogens (Leak 1998).

There are two key stages in the interaction of some trypanosomes (e.g. *Trypanosoma brucei* or *T. congolense*) with tsetse flies, namely the initial establishment of infection in the midgut and the subsequent maturation of the trypanosomes to produce infective stages in the salivary glands or oesophagus (Aksoy, Gibson & Lehane 2003). The current study was therefore conducted to identify the principle vector of trypanosomes in both field and controlled experimental conditions by comparing the vector competence of *G. austeni* and *G. brevipalpis* in transmitting *T. congolense* to cattle.

Materials and methods

Field collection of tsetse flies

Glossina brevipalpis and *G. austeni* were collected between 2006 and 2008 from areas known to be endemic with nagana in north-eastern KwaZulu-Natal (Figure 1). Flies were collected using the H-trap designed for collecting these two species (Kappmeier 2000). Traps were baited with a mixture of 1-octen-3-ol and 4-methylphenol, dispensed from heat-sealed sachets in the presence of acetone. The traps were emptied daily and flies were transported to the Agricultural Research Council (ARC) Field Research Station at Kuleni in a cooler box. Collected flies were divided into two groups, of which one was immediately used to determine the infection rate and the other group was used in xenodiagnosis experiments. Flies were couriered to the ARC-Onderstepoort Veterinary Institute (OVI) laboratory, situated 600 km from the collection areas.

Determination of infection rates in tsetse flies

A total of 442 *G. brevipalpis* and 40 *G. austeni* specimens were dissected immediately after collection and examined for trypanosome infections. An additional 195 *G. brevipalpis* specimens were dissected after transmission experiments to confirm the results of the infectivity testing in cattle. Dissections were conducted according to the method of Lloyd and Johnson (1924) and as described by Leak, Ejigu and Vreysen (2008). Flies were dissected to expose immature infections (non-infective trypanosome forms) in the midgut and mature infections (infective trypanosome forms) in the proboscis (Jordan 1976; Van den Bossche *et al.* 2004). The respective organs were placed onto microscope slides containing droplets of phosphate-buffered saline (with

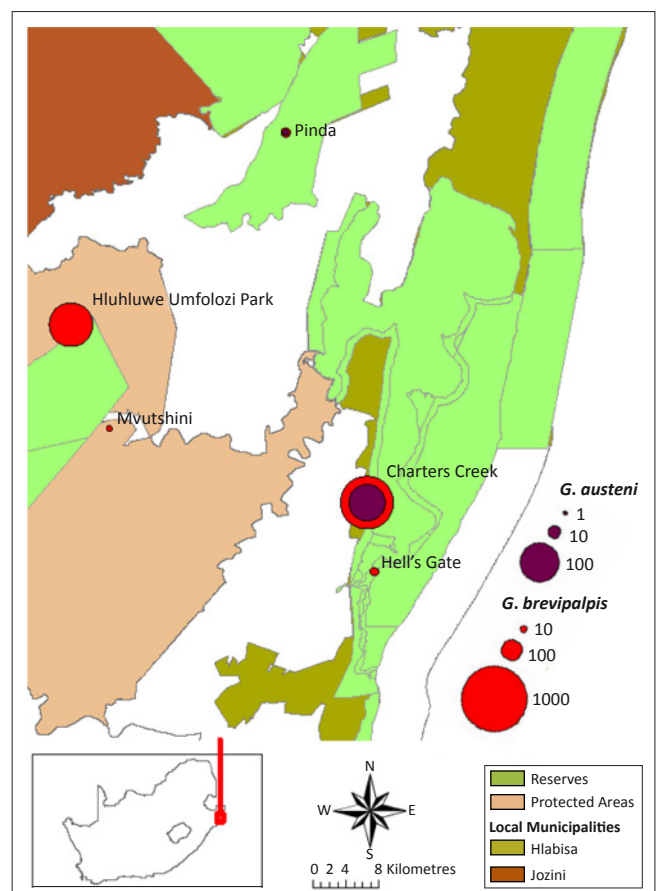
glucose) and covered with cover slips. Infection rates were determined by direct observation of prepared slides under a compound microscope using a 10× eyepiece and a 25× objective.

Maintenance of tsetse flies

At the ARC-OVI, field-captured flies were maintained under the same conditions as the colony-reared flies. The ARC-OVI houses well-established colonies of *G. austeni* and *G. brevipalpis*. These colonies were established in 2002 with seed materials of *G. austeni* obtained from the Tsetse and Trypanosomosis Research Institute in Tanga, Tanzania and *G. brevipalpis* supplied by the Insect Pest Control Sub-Programme of the International Atomic Energy Agency (IAEA) laboratories in Seibersdorf, Austria. Colony flies were maintained at 75% relative humidity and 24 °C and fed on artificial membranes using high-quality gamma-irradiated bovine blood according to the standard operating procedure of the Food and Agriculture Organization/IAEA (2006).

Experimental infection of colony flies using infected animals

The artificial infection of colony flies was carried out to compare the results with the infection rate in field-collected



Source: Map courtesy of Chantel de Beer, Dept of Entomology, Onderstepoort Veterinary Institute

The purple and the red circles represents the number of *Glossina austeni* and *Glossina brevipalpis* flies caught at a particular site ranging between 1 and 100 and 10 and 1000 respectively.

FIGURE 1: Location of collection sites for *Glossina brevipalpis* and *Glossina austeni*.



flies. Each of four susceptible cattle was infected intravenously with (1×10^6) organisms using four different *T. congolense* isolates. Three of the isolates (I, II and BmrngK2) were isolated from cattle at Boomerang Farm and the fourth (MVU10) from cattle at the Mvutshini community dip tank. All isolates were maintained in mice. Molecular characterisation of all four isolates, according to a method described by Geysen, Delespau and Geerts (2003), revealed that they belonged to *T. congolense*. The infected cattle were monitored daily for the development of trypanosome parasitaemia and fever, whilst packed cell volume (PCV) values were also determined. Flies were fed on cattle when parasites were detected in the buffy coat preparations. Experimental animals were clipped on their flanks and localised areas shaved with a scalpel blade to expose the skin (Akol & Murray 1983). Flies were confined in cages in groups of not more than 20 and allowed to feed until fully engorged. The engorged flies were maintained on artificial feeding membranes (as described for the colony flies) for 21 days before dissection (Masumu *et al.* 2006).

Experimental infection of susceptible animals using field-collected flies

To demonstrate the infectivity of field-collected flies in cattle, 10 batches of *G. brevipalpis* (699 flies in total) and four batches of *G. austeni* (49 flies in total) were used in the transmission experiments. The batch sizes were determined by the number of flies captured in an area on a specific date. For each area the number of collections ranged from 16 to 180 and 6 to 40 for *G. brevipalpis* and *G. austeni*, respectively. Flies were fed as mentioned earlier. Where the number of flies per batch exceeded 20, more cages were used. Each batch was allowed to feed on a susceptible Nguni bovine, except for one batch of *G. austeni*, which was fed on a goat. The animals were monitored daily for the development of trypanosome parasitaemia, fever and changes in PCV over 30 days. Animals considered anaemic (i.e. PCV = 18% for three consecutive days) received treatment with 3.5 mg/kg diminazene aceturate (Berenil™).

Statistical analyses

Variation in infection prevalence between isolates and tsetse flies was analysed using the statistical program GenStat® (Payne *et al.* 2007). Testing was performed at the 5% significance level, with $p < 0.05$ used as the cut-off for statistical significance.

Ethical considerations

Ethical approval for the experiments was obtained from the Animal Ethics Committee of the OVI (ref. 07/20/C174)

and Animal Use and Care Committee of the University of Pretoria, Faculty of Veterinary Sciences (ref. VO56-09).

Results

A total of 637 *G. brevipalpis* and 40 *G. austeni* specimens collected from the field were dissected and their infection prevalence assessed (Table 1). Analysis revealed 8% immature and mature trypanosome infections the midgut and proboscis of *G. austeni*. On the other hand, only 1% of immature infections were detected in the midgut of *G. brevipalpis*.

To confirm the infection rate results obtained for the field-collected tsetse flies, laboratory-controlled fly infectivity experiments were conducted. A total of 534 colony-reared *G. austeni* and 882 *G. brevipalpis*, fed on experimentally infected parasitaemic cattle, were dissected. Dissection results indicated that all four isolates (BoomerangI, BoomerangII, BmrngK2 and MVU10) became established in the midgut of both *G. austeni* (20% – 33%) and *G. brevipalpis* (1% – 4%) (Table 2). Of the infected flies, 22% ($n = 121$) immature infections and 4% ($n = 21$) mature infections were found in *G. austeni* fed on four cattle infected with different *T. congolense* isolates. Only 2% ($n = 17$) immature infections were seen in *G. brevipalpis* and no parasites were detected in the proboscis.

Vector competence was also assessed to confirm the results on the infection rates with trypanosomes in *G. brevipalpis* and *G. austeni* collected from game parks and communal dip tanks in KwaZulu-Natal. The infectivity of *G. brevipalpis* fed on susceptible cattle under controlled conditions was not shown and no trypanosome transmission was observed from any of the flies (as many as 180 per animal). On the other hand, transmission with *G. austeni* was achieved with a small number of feeding flies (i.e. fewer than 10 per animal).

There were no significant differences in the immature infection prevalence between the four isolates in *G. austeni* ($p = 0.158$). However, there was a significant difference ($p = 0.025$) in the immature infection prevalence of these isolates in *G. brevipalpis*.

A higher number of midgut infections were observed to have resulted from BoomerangI and II (4.3% and 2.6%, respectively) than from MVU10 and BmrngK2 (0.36% and 0.50%, respectively), as shown in Table 3. In contrast, the number of *G. austeni* with mature infections from the four isolates differed significantly ($p = 0.007$). Flies infected with

TABLE 1: Prevalence of trypanosome parasites in midgut and proboscis of field-collected *Glossina austeni* and *Glossina brevipalpis*.

Experimental tsetse groups	<i>Glossina austeni</i>		<i>Glossina brevipalpis</i>		p-value
	N	%	N	%	
Number dissected	40	–	637	–	–
Number of flies with immature infection (midgut)	3	8	5	1	0.009
Number of flies with mature infection (proboscis)	3	8	0	0	< 0.001

N, sample size.

p-values < 0.05 were used as the cut-off for statistical significance.



BoomerangI and II isolates had a higher infection prevalence (9.5% and 8.2%, respectively) compared with those infected with isolates MVU10 and BmrngK2 (2.6% and 1.6%, respectively) (Table 3). Mature infections were not detected in the proboscis of any *G. brevipalpis* flies.

After the flies had fed on the animals, parasites were detected in blood samples within 14, 17 and 22 days for cattle and 15 days for the goat. All animals subsequently presented with clinical signs of anaemia (Table 4).

Discussion

In the present study, only 1% of *T. congolense* infections were found in the midgut of *G. brevipalpis*. In contrast, the infection rate in the midgut and the proboscis of *G. austeni* was significantly higher, with 8% detected in both organs. The age structure of the two *Glossina* species had not been determined in the field-collected flies. It has been reported that older flies are more likely to be infected than younger flies (Harley 1966; Jordan 1976; Woolhouse *et al.* 1994). The factors affecting the trypanosome infection rate in flies and contributing to their being refractory to infection have been detailed and discussed by several authors (Jordan 1976; Roditi & Lehane 2008; Welburn & Maudlin 1999). Factors such as age may influence the readiness with which flies can be infected. According to Harley (1967), the longer the female of some fly species, such as *G. brevipalpis*, lives, the more likely she is to be infected with *T. congolense*. In contrast, species such as *G. austeni* can be readily infected when they are 1 day old and less successfully later (Jordan 1976; Ward 1968). In our study, both *G. austeni* and *G. brevipalpis* colony specimens were fed a day after emergence and were able to

establish infections in the midgut. However, trypanosomes could not develop to maturity in *G. brevipalpis*.

The results showing the poor efficiency of *G. brevipalpis* as a vector was unexpected based on their higher population densities in areas close to the Hluhluwe–Umfolozzi Game Reserve where high infection prevalence in cattle had been reported (Gillingwater, Mamabolo & Majiwa 2010; Van den Bossche *et al.* 2006). These results further suggest that *G. austeni* is the major vector of trypanosomes in the area, despite its relatively low population density as reported by Esterhuizen *et al.* (2005) and Hendrickx *et al.* (2003). The relatively low numbers of *G. austeni* collected in this study indicate either that the natural population density is low or that the H-trap is not effective for the collection of this species. Similar observations were reported by Gaturaga, Maloo and Loehr (1989) when they collected only 33 flies representing two *Glossina* species over a period of one year, despite a high trypanosome infection rate (22%) amongst cattle. They attributed the low numbers of *G. austeni* collected, assumed to be the major vector, to the inefficiency of the biconical trap used.

Interestingly, flies infected with isolates BoomerangI and II exhibited more midgut infections in *G. brevipalpis* and more mature infections in *G. austeni* than those infected with isolates MVU10 and BmrngK2. Both fly species seemed to be more susceptible to infection with Boomerang I and II isolates; however, *G. brevipalpis* is refractory to subsequent parasite maturation occurring in the proboscis. Goossens *et al.* (2006) found a very low prevalence of *T. congolense* in cattle (0.8%) on Mafia Island, Tanzania, where *G. brevipalpis* is the only

TABLE 2: Trypanosome infection rate in midgut and proboscis of *Glossina austeni* and *Glossina brevipalpis* colony flies fed on infected cattle (calculated *p*-values).

Isolate	Experimental tsetse groups	<i>Glossina austeni</i>		<i>Glossina brevipalpis</i>		<i>p</i> -value
		<i>N</i>	%	<i>N</i>	%	
BoomerangI	Number dissected	63	–	163	–	–
	Flies with infection in midgut	21	33	7	4	< 0.001
	Flies with infection in proboscis	6	10	0	0	< 0.001
BoomerangII	Number dissected	85	–	274	–	–
	Flies with infection in midgut	20	24	7	3	< 0.001
	Flies with infection in proboscis	7	8	0	0	< 0.001
MVU10 (Mvutshini)	Number dissected	193	–	247	–	–
	Flies with infection in midgut	42	22	1	0	< 0.001
	Flies with infection in proboscis	5	3	0	0	0.016
BmrngK2 (Boomerang)	Number dissected	193	–	198	–	–
	Flies with infection in midgut	38	20	2	1	< 0.001
	Flies with infection in proboscis	3	2	0	0	0.119
	Total midgut infections	121/534	22	17/882	2	–
	Total proboscis infections	21/534	4	0	–	–

TABLE 3: Infection prevalence in the midgut and proboscis of infected colony *Glossina austeni* and *Glossina brevipalpis* flies.

Species	Stage	Isolate				<i>p</i> -value
		BoomerangI (%)	BoomerangII (%)	MVU10 (Mvutshini) (%)	BmrngK2 (Boomerang) (%)	
<i>Glossina austeni</i>	Immature	33	24	22	20	0.1582
	Mature	10 ^a	8 ^a	3 ^b	2 ^b	0.0072
<i>Glossina brevipalpis</i>	Immature	4 ^a	3 ^a	0 ^b	1 ^b	0.0254
	Mature	0	0	0	0	–

^a, higher infections.

^b, lower infections.



tsetse fly species encountered and widely distributed. They attributed the low prevalence to a combination of factors, such as frequent use of prophylactic treatment of cattle with trypanocidal drugs, a low feeding frequency of *G. brevipalpis* on cattle and the low vectorial capacity of the fly. In contrast, Wilson, Dar and Paris (1972) found the infection rate with *T. congolense* in field-collected *G. brevipalpis* to be about 2% in their study in Uganda. The injection of the infected proboscis collected from these flies produced patent infection in mice and thus demonstrated the ability of *T. congolense* to mature in the proboscis.

The present data do not support results from two recent studies on the trypanosomes infection rates in tsetse flies conducted in the same area using only polymerase chain reaction (PCR) analysis (Gillingwater *et al.* 2010; Mamabolo *et al.* 2009). Mamabolo *et al.* (2009) detected trypanosome DNA in 89% of the flies examined but the results did not specify the species origin. Furthermore, this molecular test does not distinguish between mature and immature infections. In the study of Mamabolo *et al.* (2009), the injection of a suspension of macerated fly proboscis did not produce any viable infections in mice that were monitored for the development of trypanosome parasitaemia. Similarly, Gillingwater *et al.* (2010) reported a higher percentage (20%) of flies testing positive for trypanosome DNA in the midgut with only 1.6% of mature infections. Again, the results were not separated according to species. The higher infection rates detected by the PCR analysis may be related to recent feeding of the flies on infected animals although these trypanosomes would not necessarily develop successfully in the midgut or the proboscis.

Conclusion

A wealth of entomological data have been collected over the years in KwaZulu-Natal, which were used by Hendrickx *et al.* (2003) to produce distribution and prediction maps. However, parallel data on the epidemiology of the disease have not been generated to support the intention of the veterinary authorities to control or eradicate tsetse flies from

South Africa. The results from the current study support the findings of Goossens *et al.* (2006) that *G. brevipalpis* is not the main vector of *T. congolense* in KwaZulu-Natal, despite its higher abundance, whereas *G. austeni* has been shown to have a higher vector competence. Focus should, therefore, be directed towards the control of *G. austeni* in the province whilst more research is still needed to develop more efficient traps to monitor the population dynamics of this species before, during and after control operations.

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Competing interests

The authors declare that they have no financial or personal relationship(s) which may have inappropriately influenced them in writing this paper.

Authors' contributions

M.M. (ARC-Onderstepoort Veterinary Institute) was the project leader and performed most of the experiments and wrote the manuscript. J.M. (Universite Pedagogique Nationale) was a student mentor who assisted in the execution of some experiments and also edited the manuscript. A.L. (ARC-Onderstepoort Veterinary Institute) was the project supervisor and also contributed to revision and editing of the manuscript. B.M. (ARC-Onderstepoort Veterinary Institute) was the co-supervisor of the study and contributed to revision and editing of the manuscript. P.v.d.B. (Institute of Tropical Medicine) was a co-supervisor to J.M. (Universite Pedagogique Nationale) with regard to providing mentorship and assistance with specific experiments.

TABLE 4: Infectivity of *Glossina austeni* and *Glossina brevipalpis* collected from different field sites and fed on susceptible hosts under controlled conditions.

Cattle number	Infection date	Fly species	Collection site	Number fed	Pre-patent period (days to treatment)†
8306	26 Nov. 2006	<i>G. austeni</i>	C. Creek	9	22 (64)
8461	28 Jan. 2007	<i>G. austeni</i>	C. Creek	14	17 (46)
755	19 Nov. 2008	<i>G. austeni</i>	C. Creek	6	14 (58)
8024 (goat)	28 Oct. 2006	<i>G. austeni</i>	C. Creek	20	15 (35)
8383	15 July 2006	<i>G. brevipalpis</i>	C. Creek	40	No infection
8379	15 July 2006	<i>G. brevipalpis</i>	Hluhluwe	40	No infection
8269	26 Nov. 2006	<i>G. brevipalpis</i>	C. Creek	100	No infection
8340	26 Nov. 2006	<i>G. brevipalpis</i>	Hluhluwe	120	No infection
8462	28 Jan. 2007	<i>G. brevipalpis</i>	C. Creek	54	No infection
8465	28 Jan. 2007	<i>G. brevipalpis</i>	Hluhluwe	50	No infection
8467	28 Jan. 2007	<i>G. brevipalpis</i>	C. Creek	64	No infection
751	01 Aug. 2008	<i>G. brevipalpis</i>	Hluhluwe	35	No infection
755	01 Aug. 2008	<i>G. brevipalpis</i>	C. Creek	180	No infection
756	01 Aug. 2008	<i>G. brevipalpis</i>	Hell's Gate	16	No infection

†, Treatment day with Berenil™; PCV dropped to 18% for 3 consecutive days.



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