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Comparison of the susceptibility of different *Glossina* species to simple and mixed infections with *Trypanosoma (Nannomonas) congolense* savannah and riverine forest types

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Abstract. Teneral *Glossina morsitans mositans, G.m.submorsitans, G.palpalis gambiensis* and *G.tachinoides* were allowed to feed on rabbits infected with *Trypanosoma congolense* savannah type or on mice infected with *T.congolense* riverine-forest type. The four tsetse species and subspecies were also infected simultaneously *in vitro* on the blood of mice infected with the two clones of *T.congolense* via a silicone membrane. The infected tsetse were maintained on rabbits and from the day 25 after the infective feed, the surviving tsetse were dissected in order to determine the infection rates.

Results showed higher mature infection rates in *morsitans*-group tsetse flies than in *palpalis*-group tsetse flies when infected with the savannah type of *T.congolense.* In contrast, infection rates with the riverine-forest type of *T.congolense* were lower, and fewer flies showed full development cycle. The intrinsec vectorial capacity of *G.m.submorsitans* for the two *T.congolense* types was the highest, whereas the intrinsic vectorial capacity of *G.p.gambiensis* for the Savannah type and *G.m.morsitans* for the riverine-forest type were the lowest. Among all tsetse which were infected simultaneously with the two types of *T.congolense*, the polymerase chain reaction detected only five flies which had both trypanosome taxa in the midgut and the proboscis. All the other infections were attributable to the savannah type.

The differences in the gut of different *Glossina* species and subspecies allowing these two sub-groups of *T.congolense* to survive better and undergo the complete developmental cycle more readily in some species than other are discussed.

Key words. *Glossina* spp., *Trypanosoma congolense*, savannah type, riverine-forest type, PCR, vectorial competence.

Introduction

Trypanosoma congolense Broden (Trypanosomatidae: Kinetoplastida) is a complex of several trypanosome infraspecific groups among which four taxa can be identified by specific DNA probes based on repeated sequences. From the nucleotide sequences of these *T.congolense* group-specific DNA, oligonucleotide primers are also available for targeting specific amplification, by polymerase chain reaction (PCR), of DNA from trypanosomes present in the tsetse organs. The savannah type of *T.congolense* (Majiwa *et al.*, 1985; Majiwa & Webster, 1987) is the most widespread type in Africa and is frequently encountered in domestic livestock, particularly cattle (Nyeko *et al.*, 1990; Reifenberg *et al.*, 1997). The riverine-forest type of *T.congolense* (Gibson *et al.*, 1988) is more restricted to the humid regions of West Africa (Young & Godfrey, 1983; McNamara & Snow, 1991; Solano *et al.*, 1995; Masiga *et al.*, 1996), but was also detected in Zambia and Zimbabwe (Woolhouse *et al.*, 1996). This group was never described in East Africa. The Kilifi type, originating from the Kenya coast, is predominant in East Africa (Majiwa & Otieno,

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1990). Nevertheless, it was also detected in Central African Republic (D'Amico, 1993), in Côte d'Ivoire (McNamara *et al.*, 1995; Masiga *et al.*, 1996), in Zimbabwe and Zambia (Woolhouse *et al.*, 1994, 1996). The Tsavo type isolated for the first time from *Glossina pallidipes* Austen (Diptera: Glossinidae) caught at the Ngulia Rhino Sanctuary, Tsavo West National Park, Kenya (Majiwa *et al.*, 1993), was never detected in other areas of Africa except once only in Central African Republic (D'Amico, 1993).

Affinity relationships between *T.congolense* subgroups and tsetse species and subspecies were suspected in several studies. In The Gambia, the riverine-forest group of *T.congolense* was only identified in *G.palpalis gambiensis* Vanderplank populations whereas the savannah type was detected exclusively in *G.morsitans submorsitans* Newstead (McNamara & Snow, 1991). Other studies also showed this apparent *T.congolense* riverine-forest type/*palpalis*-group tsetse flies association in Zaire (Schares & Melhitz, 1996) and Cameroun (Morlais, pers. comm.). Nevertheless, the concomitant presence of the above two genotypic groups was described in *G.longipalpis* Wiedemann in Côte d'Ivoire (Solano *et al.*, 1995), in *G.pallidipes* in Zimbabwe (Woolhouse *et al.*, 1996) and in *G.tachinoides* Westwood in Burkina Faso (Reifenberg, 1996). These observations provide further evidence that such associations exist, but probably depend on vector and parasite factors as well as ecological factors.

The present study was undertaken to determine experimentally the susceptibility of four tsetse species and subspecies belonging to the two taxonomic groups, namely *morsitans* and *palpalis*, to single and mixed infections with the savannah and the riverine-forest types of *T.congolense*, in order to study in laboratory conditions the associations suggested by field observations.

Materials and Methods

Tsetse. Glossina morsitans submorsitans, G.palpalis gambiensis and *G.tachinoides* used in this study originated from Burkina Faso, and *G.morsitans morsitans* from Zimbabwe. All the flies were from the CIRAD-EMVT/ORSTOM colonies bred in Montpellier.

Trypanosomes. T.congolense clone E325 (savannah type) was isolated from infected wild *G.pallidipes* caught in Uganda (Uilenberg *et al.*, 1973). *T.congolense* clone DINDERESSO/80/CRTA/3 (riverine-forest type) was isolated from a naturally infected dog in Burkina Faso (Duvallet *et al.*, 1993).

Single clone transmissions. T.congolense E325 and DINDERESSO/80/CRTA/3 stabilates were thawed and 0.5 ml were injected intravenously into a rabbit and intraperitoneally into mice, respectively. The riverine-forest type of *T. congolense* we used was propagated in mice since this clone produced only transient parasitaemia in rabbits.

The infection was monitored by bleeding the rabbit from the ear and the mice from the tail. The blood was examined for parasites by phase-contrast microscopy at ×400 magnification.

After the parasites were detected during the first parasitaemic wave (10^6 trypanosomes/ml or greater, *G.m.morsitans, G.m.submorsitans, G.p.gambiensis* and *G.tachinoides* teneral flies destined to be infected with *T.congolense* savannah type were allowed to feed on the ears of the infected rabbit. Teneral flies belonging to the four tsetse species or subspecies destined to be infected with *T.congolense* riverine-forest type were fed on the belly of five infected mice. Tsetse were given a single infective feed and were maintained on clean rabbits. Infected mice were killed and the blood from these mice was cryopreserved in liquid nitrogen. Infected rabbits were treated with trypanocidal drug (3.5 mg/kg body weight of Berenil®) to prevent death. 25 days after the initial infective bloodmeal the surviving tsetse were given their last meal and then starved for 2 days to reduce or eliminate partially digested blood and thus facilitate dissection of the gut and observation of trypanosomes. Flies were dissected and their midguts, labra and hypopharynges were examined for the presence of trypanosomes with a phase-contrast microscope.

Mixed savannah/riverine-forest types transmissions. Each *T.congolense* clone was grown in five mice and after the first parasitaemic wave, blood was collected by cardiac puncture in heparin and then was mixed with uninfected mouse blood to produce a final concentration of each *T.congolense* clone of 10^5/ml in the final mixture (concentration evaluated with a Neubauer haemocytometer). Teneral flies of each species were given a single *in vitro* infective feed through a silicone membrane. Following completion of infected feeds, tsetse were maintained on uninfected rabbit and from the day 25 after the infected bloodmeal, the surviving tsetse were dissected, and their midguts and proboscises were examined for the presence of trypanosomes with a phase-contrast microscope. Infected organs (midguts and proboscis) identified by microscopy were transferred to microcentrifuge tubes and prepared according to the following protocols.

Preparation of crude templates for PCR. Tsetse midguts were prepared according to a recent protocol (Penchenier *et al.*, 1996). Infected midguts were put into 1.5 ml microcentrifuge tubes containing 500 µl of sterilized and nuclease free water. Tubes were vortexed every 1–2 min at room temperature. After centrifugation (2 min at 15,000 rpm) in a microcentrifuge, supernatants were discarded and 50 µl of resin added (Ready AMP genomic purification kit, Madison, Wis.,USA). Pellets were resuspended and incubated for 20 min at 56°C, vortexed and then kept at 100°C for 10 min. After 2 min of centrifugation at 15,000 rpm, 3 µl of supernatant liquid containing single-stranded DNA were used for PCR.

The proboscis of each infected fly was placed in 50 µl of sterilized water; after a brief agitation at room temperature, 3 µl were used in a PCR reaction.

Primers. Oligonucleotide primers psecific for the *T.congolense* savannah and riverine-forest types (Masiga *et al.*, 1992) were kindly provided by CIRAD-EMVT Montpellier, France.

PCR cycling. Standard PCR amplifications were carried out in 25 µl reaction mixtures containing, as final concentrations, 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 200 µM of each of the four desoxyribonucleotide triphosphates (dNTPs), primers at 100 ng/ml, 3 µl DNA template and 1.25 units of Taq polymerase (HITAQ, Bioprobe). The reaction mixtures were overlaid with paraffin oil to prevent evaporation. Reactions involved 30 cycles of denaturation at 94°C for 1 min, annealing at 56°C for 1 min and extension at 72°C for 1 min. At the end of the last cycle, the extension reaction was continued at 72°C for 5 min before cooling to 4°C. From each sample, 15 µl was then electrophoresed through 2% agarose and amplification products revealed by ultraviolet transillumination.

Results

Single clone transmissions

Infections were scored as immature if they were found in the gut only. They were classified as mature if they were found in the midgut and labrum or the midgut, labrum and hypopharynx. Actually, according to previous studies, we considered that labral infections with *T.congolense* are likely to be accompanied by infection of the hypopharynx (Clarke, 1965) and that transformation to metacyclic forms may not strictly be limited to the hypopharynx (Thévenaz & Hecker, 1980).

All the infection rate data were analysed using the chi-square test. The infections of tsetse were quantified (Intrinsic Vectorial Capacity) (Le Ray, 1989).

The infection prevalences in male and female *Glossina* spp. with *T.congolense* Savannah type are given in Table 1. Infection prevalene in the midgut of *morsitans*-group tsetse flies (*G.m.morsitans* and *G.m.submorsitans*) and *palpalis*-group tsetse flies (*G.p.gambiensis* and *G.tachinoides*) were not statistically different ($\chi^2 = 0.8$, $P > 0.05$). Infection prevalence in the labrum were significantly higher in *morsitans*–group tsetse flies than in *palpalis*-group tsetse flies ($\chi^2 = 168.3$, $P < 0.005$).

There was no significant differences between the two sexes of *G.m.morsitans* and *G.m.submorsitans*. The infection prevalences in labrum of *G.p.gambiensis* were higher in male than in female ($\chi^2 = 32.5$, $P < 0.005$), and in the midgut of *G.tachinoides* were higher in female than in male ($\chi^2 = 7.5$, $P < 0.005$).

The IVC varied from 0.809 in *G.m.submorsitans* to 0.082 in *G.p.gambiensis* (Table 4).

Table 2 shows that infection rates by *T.congolense* riverine-forest type in the midgut of tsetse flies were significantly higher in *palpalis*-group than in *morsitans*-group ($\chi^2 = 16.6$, $P < 0.005$). The differences between labrum infection rates did not differ statistically.

No significant differences were found in the infection prevalences between male and female of *G.tachinoides* and *morsitans*-group flies. Infection prevalences in the midgut of female were higher than in male in *G.p.gambiensis* ($\chi^2 = 18.1$, $P < 0.005$).

Table 1. Infection prevalences in different *Glossina* species when fed on a rabbit infected with *Trypanosoma congolense* clone E325 (savannah type).

Tsetse species	Sex of tsetse	No. of tsetse dissected	Infection prevalence (%)			Immature infection prevalence (%)
			Midgut	Labrum	Hypopharynx	
G.m.morsitans	Male	51	41.2	35.3	35.3	14.3
	Female	68	58.8	45.6	39.7	22.5
	Total	119	51.2	41.2	37.8	19.7
G.m.submorsitans	Male	47	91.9	89.2	81.1	2.3
	Female	84	86.9	76.2	69	12.3
	Total	131	88.5	80.9	73.3	8.6
G.p.gambiensis	Male	64	75	25	7.8	66.6
	Female	180	78.3	2	0	97.1
	Total	244	77.4	8.2	2	89.4
G.tachinoides	Male	36	50	16.6	8.3	66.6
	Female	96	75	21.9	7.3	70.8
	Total	132	68.2	20.4	7.6	70

Table 2. Infection prevalences in different *Glossina* species when fed on mice infected with *Trypanosoma congolense* clone DINDERESSO/80/CRTA/3 (riverine-forest type).

Tsetse species	Sex of tsetse	No. of tsetse dissected	Infection prevalence (%)			Immature infection prevalence (%)
			Midgut	Labrum	Hypopharynx	
G.m.morsitans	Male	74	10.8	1.3	1.3	87.5
	Female	144	11.1	2.1	2.1	81.2
	Total	218	11	1.8	1.8	83.3
G.m.submorsitans	Male	45	48.8	8.8	6.6	81.8
	Female	73	42.4	9.6	9.6	77.4
	Total	118	44.9	9.3	8.5	79.2
G.p.gambiensis	Male	99	34.3	5	4	85.3
	Female	99	64.6	3	3	95.3
	Total	198	49.5	4	3.5	91.8
G.tachinoides	Male	56	16	5.3	1.8	66.6
	Female	92	23.9	4.3	1.1	81.8
	Total	148	20.9	4.7	1.3	77.4

Table 3. *Infection prevalences in different Glossina* species infected simultaneously with *Trypanosoma congolense* clone E325 (Savannah type) and clone Dinderesso/80/CRTA/3 (riverine-forest type).

Tsetse species	Sex of tsetse	No. of tsetse dissected	Infection prevalence (%)			Immature infection prevalence (%)
			Midgut	Labrum	Hypopharynx	
G.m.morsitans	Male	35	20	20	20	0
	Female	43	27.9	27.9	27.9	0
	Total	78	24.3	24.3	24.3	0
G.m.submorsitans	Male	52	69.2	69.2	69.2	0
	Female	47	53.2	53.2	53.2	0
	Total	99	61.6	61.6	61.6	0
G.p.gambiensis	Male	0	–	–	–	–
	Female	22	18.1	0	0	100
	Total	22	18.1	0	0	100
G.tachinoides	Male	45	40	13.3	8.8	66.6
	Female	34	58.8	11.7	8.8	80
	Total	79	48.1	12.6	8.8	73.7

The IVC varied from 0.093 in *G.m.submorsitans* to 0.027 in *G.m.morsitans* (Table 5).

The parasite loads in the labra of infected tsetse were generally very high with *T.congelense* Savannah type and low with *T.congolense* riverine-forest type.

Mixed savannah/riverine-forest types transmissions

The infection prevalences in the four species and subspecies of tsetse flies infected simultaneously with *T. congolense* savannah and riverine-forest types are given in Table 3.

Only 22 to 146 *G.p.gambiensis* were successfully engorged via silicone membrane since the CIRAD-EMVT colony flies appeared to be more refractory than the other species to this artificial method of infection.

Table 4. Intrinsic vectorial capacity (IVC) in *G.m.morsitans*, *G.m.submorsitans*, *G.p.gambiensis* and *G.tachinoides* infected by *T.congolense* E325 (savannah type).

Tsetse species	IVC
G.m.morsitans	0.411
G.m.submorsitans	0.809
G.p.gambiensis	0.082
G.tachinoides	0.204

Table 5. Intrinsic vectorial capacity (IVC) in *G.m.morsitans*, *G.m.submorsitans*, *G.p.gambiensis* and *G.tachinoides* infected by *T.congolense* Dinderesso/80/CRTA/3 (riverine-forest type).

Tsetse species	IVC
G.m.morsitans	0.027
G.m.submorsitans	0.093
G.p.gambiensis	0.040
G.tachinoides	0.047

All the tsetse were found to be infected with a single *T.congolense* taxa (savannah type), except five flies (one *G.m.morsitans* and four *G.m.submorsitans*) which had both trypanosome taxa in the gut and the proboscis, according to the PCR analysis results.

The PCR failed to identify seven (5.7%) midgut infections (no amplification occured). Of these seven infected flies, four harboured trypanosomes in the proboscis which were identified by PCR as *T.congelense* savannah type.

No significant difference in infected midguts was found between *morsitans*-group and *palpalis*-group flies ($\chi^2 = 0.3$, $P > 0.05$), but the percentages of labral infections were significantly higher in the two *morsitans* subspecies than those in *palpalis*-group flies ($\chi^2 = 36.5$, $P < 0.005$).

The differences between the two sexes were not statistically different except in the midguts in *G.m.submorsitans* male compared with those in females ($\chi^2 = 7.1$, $P < 0.005$). The IVC was the highest in *G.m.submorsitans* (0.616) and the lowest in *G.tachinoides* (0.126) (Table 6).

Of the total of 784 parasitologically positive flies (single and mixed transmissions combined), we found a tendency for a greater percentage of infected females (52.7%) than infected males in the midgut (40.84%) ($\chi^2 = 19.2$, $P < 0.005$), but labral infections were significantly more numerous among males (24.4%) than

Table 6. Intrinsic vectorial capacity (IVC) in *G.m.morsitans*, *G.m.submorsitans*, *G.p.gambiensis* and *G.tachinoides* infected simultaneously by *T.congolense* E325 (savannah type) and *T.congolense* Dinderesso/80/CRTA/3 (riverine-forest type). The precise nature of the infection was not considered in the IVC calculation (according to the PCR results, all the infections were attributed to the savannah type of *T.congolense* except five flies which harboured mature mixed infections).

Tsetse species	IVC
G.m.morsitans	0.243
G.m.submorsitans	0.616
G.p.gambiensis	0.181
G.tachinoides	0.216

among females (19.98%) ($\chi^2 = 4.8$, $P < 0.005$). *Palpalis*-group tsetse (55.7%) were significantly more susceptible in the midgut than *morsitans*-group tsetse (43.8%) ($\chi^2 = 24.2$, $P < 0.005$). In turn, infection rate in the labrum of *morsitans*-group tsetse (32.82%) was higher than those in palpalis-group (10.8%) ($\chi^2 = 122.5$, $P < 0.005$). Consequently, taking into account that labral forms should presumably migrate to the hypopharynx (Clarke, 1965), we can say that immature infections were quite higher in *palpalis*-group tsetse (44.83%) than in *morsitans*-group (10%) ($\chi^2 = 261.9$, $P < 0.005$).

Discussion

This study confirmed the higher susceptibility of *morsitans*-group tsetse to infection with *T.congolense* compared to *palpalis*-group flies. Results here are in agreement with other laboratory studies (Harley & Wilson, 1968; Moloo & Kutuza, 1988) and field studies (Jordan,1964; Roberts & Gray, 1972). Our data showed that almost all infections in *G.m.submorsitans* and *G.m.morsitans* were mature, whereas the majority of the infections in *G.tachinoides* and *G.p.gambiensis* were immature, with trypanosomes present only in the gut of the flies. From the low percentages of infections of the gut alone and the gut and the proboscis in *G.p.gambiensis* and *G.tachinoides*, it appears that *palpalis*-group tsetse are less susceptible to infection by trypanosomes of the two *T.congolense* types. Since the full cyclical development of *T.congolense sensu lato* to metacyclics in tsetse should theoretically be finished by day 25, it would appear that the cyclical development of *T.congolense* of both the clones was arrested in the midgut of most tsetse belonging to the *palpalis*-group.

Tsetse from CIRAD-EMVT breeding are not used to feeding via silicone membrane, since all the colony is depending of host animals (rabbits) for *in vivo* feeding. We noticed that *G.palpalis gambiensis* flies were more refractory than the other tsetse species to the artificial method of feeding, whereas those of IAEA (Vienna, Austria) and CIRDES (Bobo-Dioulasso, Burkina Faso) are routinely fed on blood through silicone membranes. Moreover, we observed a noticeable difference in the behaviour of starved flies belonging to the *G.m.morsitans*, *G.m.submorsitans* and *G.tachinoides* species which were more aggressive than starved flies belonging to the *G.p.gambiensis* species. Such observations suggested differences in the adaptibility of feeding behaviour between different tsetse fly species in the laboratory.

The originality of this study relies on the comparison of different laboratory strains of *Glossina* species infected by the two genotypic types of *T.congolense* known to be responsible for the most important *T.congolense* disease affecting bovines in West Africa. Our results has demonstrated that the savannah type clone can become established and matured very readily in *G.m.morsitans* and more particularly in *G.m.submorsitans*. It is the reason why the pair *G.morsitans* ssp./*T.congolense* E325 was widely used for laboratory studies on the relationships between tsetse and trypanosomes (Gidudu *et al.*, 1995, 1996; Reifenberg *et al.*, 1996). This high susceptibility could not be attributed to the geographical origin of *G.m.submorsitans* (Burkina Faso) nor to the origin of the *T.congolense* stock (Uganda).

Infection prevalences found with the riverine-forest type do not confirm rigorously the affinity relationships suspected between *palpalis*-group tsetse and this type of *T.congolense*. High gut infection prevalences were obtained in *G.p.gambiensis* as well as in *G.m.submorsitans*. Very few mature infections were generally monitored, suggesting that the susceptibility of the tsetse for this *T.congolense* clone is probably due, for the most part, to the interactions with RLO (rickettsia-like organisms) known to play an important role in parasite establishment and differentiation in vectors (Welburn & Maudlin, 1989, 1990; Maudlin & Welburn, 1994).

The influence of the sex of the tsetse in infection rates did not appear clearly in this experiment. Overall results indicate that males are more susceptible than females (mature infections). Other studies undertaken with different tsetse strains and *T.congolense* stocks (Distelmanns *et al.*, 1982; Moloo *et al.*, 1992) confirmed this tendency. Maudlin *et al.* (1991) found that males were as susceptible as females to two *T.congolense* stocks, whereas males were more infected than females with several *T. brucei* subspecies. The contradictory results show that sex may play an important function in the infection prevalences which depend also on the genome of the tsetse (Distelmans *et al.*, 1985), the trypanosome stocks and the conditions (like temperature) used to infect tsetse in the laboratory.

The PCR failed to identify seven midgut infections which probably reflect the consequence of residual inhibitions of amplification persisting in purified samples. Four tsetse among the seven presumably infected at least with the savannah type of *T.congolense*, since the proboscis from these tsetse gave the corresponding specific amplification signal by PCR.

Simultaneous infections with the two types of *T.congolense* failed to corroborate some field observations showing the concomitant presence of the savannah and the riverine-forest types frequently encountered in the same fly (Solano *et al.*, 1995; Woolhouse *et al.*, 1996). The experimental conditions used to superinfect flies via silicone membrane could explain the exclusion of the riverine-forest type in the majority of the tsetse. But preliminary tests showed successful single infections with this clone of *T.congolense* in the same *in vitro* conditions (results not published). Another possible reason to explain the riverine-forest type exclusion is a competition which could occur in the vector gut between the two types of *T.congolense* ingested simultaneously by the tsetse. The causes for this are unknown, but owing to the fact that mixed infections prevailed in wild tsetse (McNamara *et al.*, 1995; Solano *et al.* 1995; Masiga *et al.*, 1996; Woolhouse *et al.*, 1996), we can conclude that artificial methods and laboratory parasites and vectors strains probably interfered with natural processes of cyclical development of trypanosomes in tsetse.

High prevalence of mixed infections in wild flies were commonly described in PCR-based field studies (McNamara *et al.*, 1995; Masiga *et al.*, 1996). Such observations are paradoxical with the difficulty in developing mixed infections in laboratory flies. This apparent discrepancy between field and laboratory data emphasizes the complexity of the tsetse receptivity for the establishment of different trypanosome species; but it also raises the problem of the cautious interpretation of PCR-based investigations on trypanosomes carried by wild flies. The PCR is a powerful tool to identify accurately trypanosomes in the different hosts, but we have to keep in mind that positive reactions may be obtained with residual traces of trypanosomes DNA.

It justifies the use of this technique as a complementary tool to microscopical methods for the trypanosome identification, since aborted infections are also likely to be detected. A recent representative example was the detection by PCR of mixed infections including *T.vivax* in *G.tachinoides* positive midguts with microscopy in Burkina Faso (Reifenberg, 1996). The *T.vivax* development cycle is restricted in the tsetse cibarial region and proboscis. Such a result probably revealed a *T.vivax* DNA specific amplification in a previous bloodmeal infected by this trypanosome species. Such a phenomenon may frequently occur in tsetse flies caught in active trypanosomosis foci. Other laboratory infections in tsetse are required to study the maturation of midgut mixed infections, in order to confirm the suitability of PCR for identifying active infections in tsetse from the field.

Further comparative studies using the different taxonomic groups of *T.congolense* and the different species and subspecies flies will also further elucidate more precisely the relationships between the *Nannomonas* subgenus trypanosomes and their tsetse vectors. Differences in the susceptibility which can be noticed can improve our knowledge about the epidemiology of *T.congolense*-trypanosomiasis in livestock in different areas of Africa.

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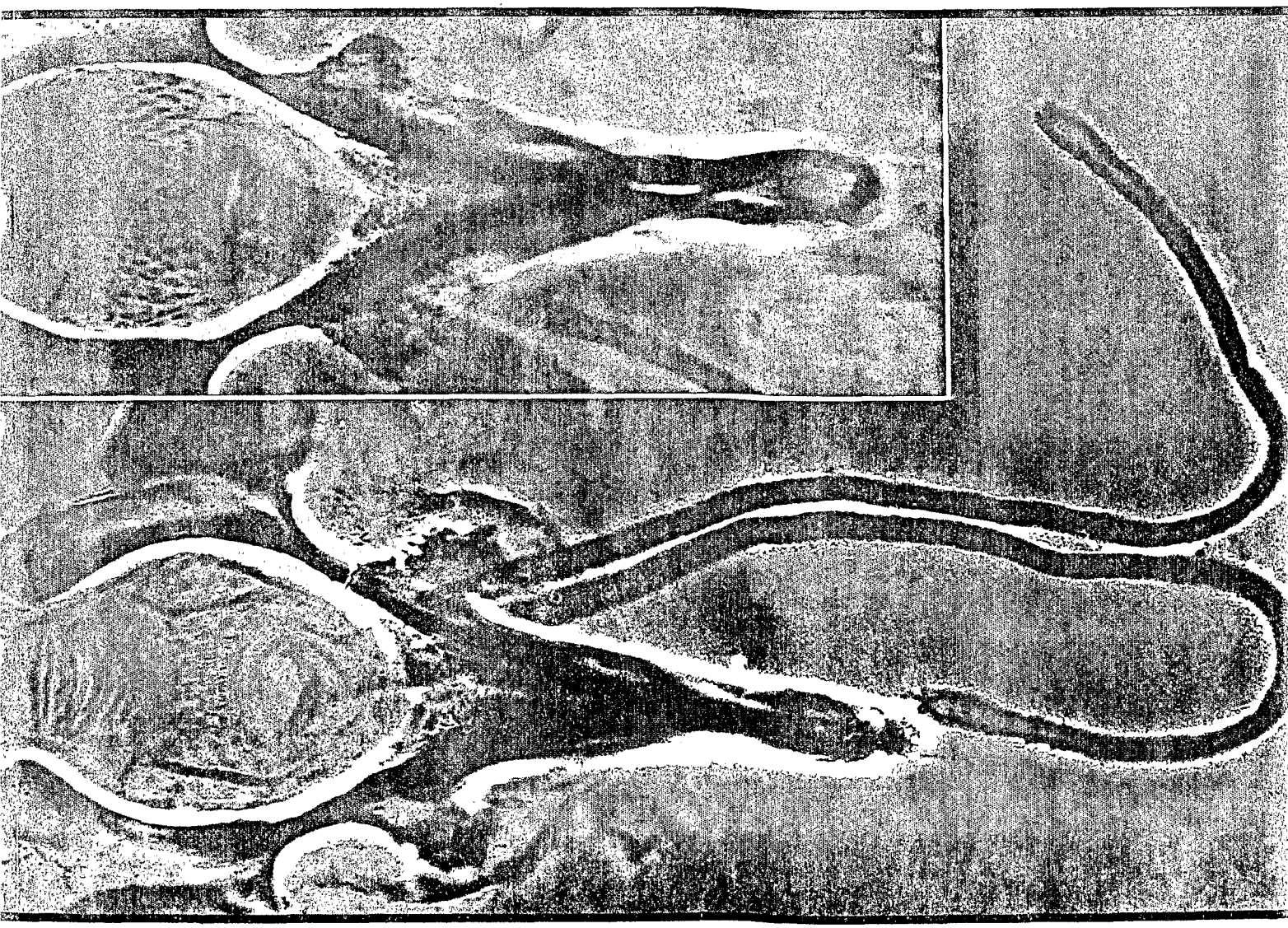

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