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Trypanosomosis prevalence in cattle on Mafia Island (Tanzania)

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Abstract

During two consecutive surveys (February and August/Sept 2002), a total of 970 cattle from the cattle population of Mafia Island (United Republic of Tanzania) were blood-sampled. All blood samples were microscopically screened for the presence of trypanosomes and a portion of these were checked for antibodies with an Ab-ELISA and for the presence of trypanosomal DNA with PCR. Microscopic evidence of trypanosomes of the *congolense* group (sub-genus Nannomonas) was found in 0.8% of the animals (8/970) and in two cases the species identified was confirmed by PCR as *Trypanosoma congolense* savannah type. Non-pathogenic *Trypanosoma theileri* were detected in 3.2% (31/970) of the samples using the Dark Ground-Buffy Coat (DG-BC) technique. For survey 1 (S1), detection of antibodies (Ab-ELISA) against pathogenic trypanosomes indicated a seroprevalence of 14.2% (68/480). Of the samples, either DG positive or with a PCV lower then 25, examined by PCR, a total of 8.4% (5/59) (selected from 970 samples), were found positive for *T. congolense*. The low prevalence of pathogenic trypanosomes on Mafia Island is intriguing, especially in view of the omnipresence of the tsetse fly *Glossina brevipalpis*. Although the presence of detected trypanosomal antibodies does not necessarily indicate a current infection, the combination of serological/parasitological examinations and the results of the PCR do support this low prevalence of trypanosomosis in cattle. Despite the low prevalence, pathogenic trypanosomes are present on Mafia Island and possible reasons for this low infection rate, taking account of the relation between *Glossina* species present, transmission risk and trypanosomes found in cattle, are discussed also in view of a future appropriate intervention strategy.

Keywords: *Glossina brevipalpis*; Tsetse flies; Tanzania; Mafia; Trypanosomosis; Sterile insect technique

1. Introduction

The elimination of the tsetse fly *Glossina austeni* Newstead from Unguja Island (Zanzibar) (1994–1997) is the first example of an area-wide integrated

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pest management programme, based on the Sterile Insect Technique (SIT) which resulted in the sustainable elimination of the disease trypanosomosis in the cattle (Vreysen et al., 2000; Saleh et al., 1999). The SIT relies on the sequential dispersal of sexually sterile male insects in high enough numbers to outcompete the native male target population. Following this successful programme, the Government of Tanzania expressed interest in applying a similar integrated approach to remove the trypanosomosis problem from Mafia Island. The feasibility of using the technique against the tsetse fly was already convincingly demonstrated in earlier programmes against *G. m. morsitans* in Tanzania (Williamson et al., 1983), against *G. palpalis gambiensis*, *G. tachinoides* and *G. m. submorsitans* in Burkina Faso (Cuisance et al., 1984; Politzar and Cuisance, 1984) and *G. palpalis palpalis* in Nigeria (Oladunmade et al., 1990). The programmes in Burkina Faso and Nigeria were not implemented according to the area-wide concept (i.e. the intervention is targeted at the *entire* pest population) and the tsetse-freed areas were quickly re-invaded after the breakdown of the artificial barriers.

The Government of Tanzania requested assistance from the International Atomic Energy Agency (IAEA) in 1999 to assess the feasibility of integrating the release of sterile males with other tsetse suppression methods to remove the vectors of the disease trypanosomosis from Mafia Island. Land use/land cover classification maps, derived from Landsat 7 Satellite imagery, were developed in the initial phase of this study to provide the basis for the development of the entomological surveys (IAEA, 1999). The entomological base line data collection was initiated in October 2001, using sticky panels, coated with the non-setting adhesive Temoocid^{R} (Vreysen et al., 1996, 1998) and H-traps (Kappmeier, 2000) baited with acetone, 4-methyl phenol, 1-octen 3-ol, and 3-*n*-propylphenol, as sampling devices. Between 2 and 6 sticky panels/traps were deployed in 16 sampling sites, which were carefully selected in different vegetation types over the entire island. The results of the survey indicated that *Glossina brevipalpis* was the only tsetse species present (i.e. sampled) and that the fly was wide-spread over the entire island but at higher densities in the northern part of the island as compared to the south. Daily catches were high in localized areas (e.g. Bweni with a maximum of 52 flies per day) and the highest fly catches were found in dense thicketed woodland (Mkamba) and forests at the western part of the island, whereas catches were much lower in sparse bushes of farmland (IAEA, 2001).

As there were no previous published data available on the prevalence of trypanosomes in cattle on Mafia Island, a survey was implemented in 2002 to collect data on the prevalence of trypanosomosis on Mafia Island to complement the entomological data. A survey on prevalence of animal diseases, conducted in the northwestern part of the island between 1988 and 1990 indicated that *Trypanosoma congolense* was a disease problem for its cattle (Mafia Veterinary Records, unpublished). This survey was part of the Heifer Project International (HPI) which was operational in the late eighties early nineties. The presence of *T. congolense* especially in the cross-breeds Holstein-Ayrshire was reported by local farmers and livestock personnel, but no written reports were available (Dr. Mueller, personal communication). This paper reports the results of two surveys carried out in 2002 to determine the prevalence of trypanosomosis in cattle on Mafia Island using parasitological, serological and PCR techniques. Limitations with funding and logistic constraints in terms of personnel and vehicles prevented a more extensive survey and an analysis of all samples using PCR and Ag-ELISA. For similar reasons, samples were not analysed using ISMM-ELISA and Diminazene-ELISA to obtain information on drug use on the island.

2. Material and methods

2.1. Study area

The district of Mafia is the largest of a group of small archipelago islands in the Indian Ocean and part of the Coastal Province of the United Republic of Tanzania (URT). Mafia Island has a total surface area of 394 km^2 and large parts of the island and surrounding sea areas are part of the Mafia Island Marine Park (MIMP), which comprises some 822 km^2. The island contains one main forest (Mrora) at the southeast side of the island and localized thickets such as Mkamba thicket on the western side of the island and remaining vegetation consists of

swamps and mangrove forest, many small forest/thicket pockets and coconut plantations (IAEA, 1999).

The human population on Mafia is estimated at 41,000 people (census 2002) mostly smallholder farmers involved in mixed farming besides fishing. Large areas of the island are planted with coconuts and cassava, rice, pigeon pea, pineapples, cashew and mango trees are typically grown on the land around the farms. The latest livestock census (1984) mentions 8500 head of cattle but projections estimate the future number of cattle to be 12,000 by the year 2005 (Atway Msangi, Personal communication). Besides the local breed, Tanzania shorthorn zebu, also their crosses with Holstein, Ayrshire and Jersey are present.

2.2. Sampling frame

The first survey was conducted at the end of the dry season/beginning rainy season (February/early March 2002) and the second survey was organised during the dry season (end of august/half September 2002). Thirteen main villages and surrounding smaller villages were selected in areas corresponding to places where traps were placed during the tsetse trapping survey in October 2001. Within the sampling frame, farms were the primary sampling unit and the animals (sometimes belonging to different owners) were considered as the sampling sub-units. All sampling areas and villages within these areas were GEO-referenced using the Global Positioning System (Garmin GPS 45). The same sampling frame was used for both surveys with the exception of the smaller island of Juani, which was not sampled in the second survey. Sampling within the village and farmer unit was done ad random. A map of Mafia Island (Fig. 1) gives an overview of the main villages and Table 1 provides Geo-referenced points for all villages where cattle were sampled.

Table 1
Area's and villages, referenced on the map (Fig. 1) and GPS-referenced data of the villages where cattle were blood sampled for Trypanosomosis during two surveys in 2002 on Mafia Island, United Republic of Tanzania

Region	GPS data
North	Bweni: 37 M 0596949-UTM 9151893 Kanga: 37 M 0597048-UTM 9144633 Jimbo: 37 M 0593432-UTM 9140072
Centre	Kirongwe: 37 M 0589712-UTM 9136130 Ndagoni: 37 M 0582947-UTM 9129507 Kungwi: 37 M 0587671-UTM 9128854 Kungwi-Mchangani: 37 M 0590039-UTM 9125974
East-Centre	Baleni: 37 M 0588430-UTM 9130131 Marimbani: 37 M 0579377-UTM 9123132
East	Utende: 37 M 0581770-UTM 9118727 Kiegeani: 37 M 0578803-UTM 9119474 Juani 37 M 0586257-UTM 9117075
South-east	Chem-chem: 37 M 0572248-UTM 9120799 Micheni: 37 M 0573566-UTM 9119236
South	Dongo-mfuruni: 37 M 0576659-UTM 9129769 Dongo: 37 M 0575064-UTM 9128211
City	Kilindoni: 37 M 0573681-UTM 9126153

2.3. Sampling and laboratory tests

Data recorded included village, name of owner, age/breed/sex and description of the animal. Age of the animal was estimated by numbers of calves born taking into account an average age at first calving of 4 years and an estimated calving interval of 2 years. Age of male animals was estimated based on number of permanent incisors and information by the farmer. Animals previously belonging to the HPI had an ear-tag, which was noted. From each animal two blood-samples were collected from the jugular vein, one into a plain vacutainer tube and one into a heparinised vacutainer. A further sample was collected from an ear vein using a heparinised micro-haematocrit capillary tube. From this sample, blood was taken in situ to prepare a thin and thick smear. All samples were placed in a cool-box for transportation to the laboratory. In the lab, Packed Cell Volume (PCV) was read using the capillary micro-haematocrit-centrifugation technique (MHCT) using the capillary containing the blood collected from the ear-vein in situ. All blood samples were examined for the presence of trypanosomes using the Dark-Ground/Buffy-Coat technique (DG-BC). Smears prepared in the field were fixed and stained using Giemsa staining for later examination. When PCV was found lower then 25 or positive for trypanosomes, 0.5 ml of the heparinised blood was inoculated into mice (67 mice were injected). From blood samples found microscopically positive for pathogenic trypanosomes (using DG-BC, blood smear or following examination

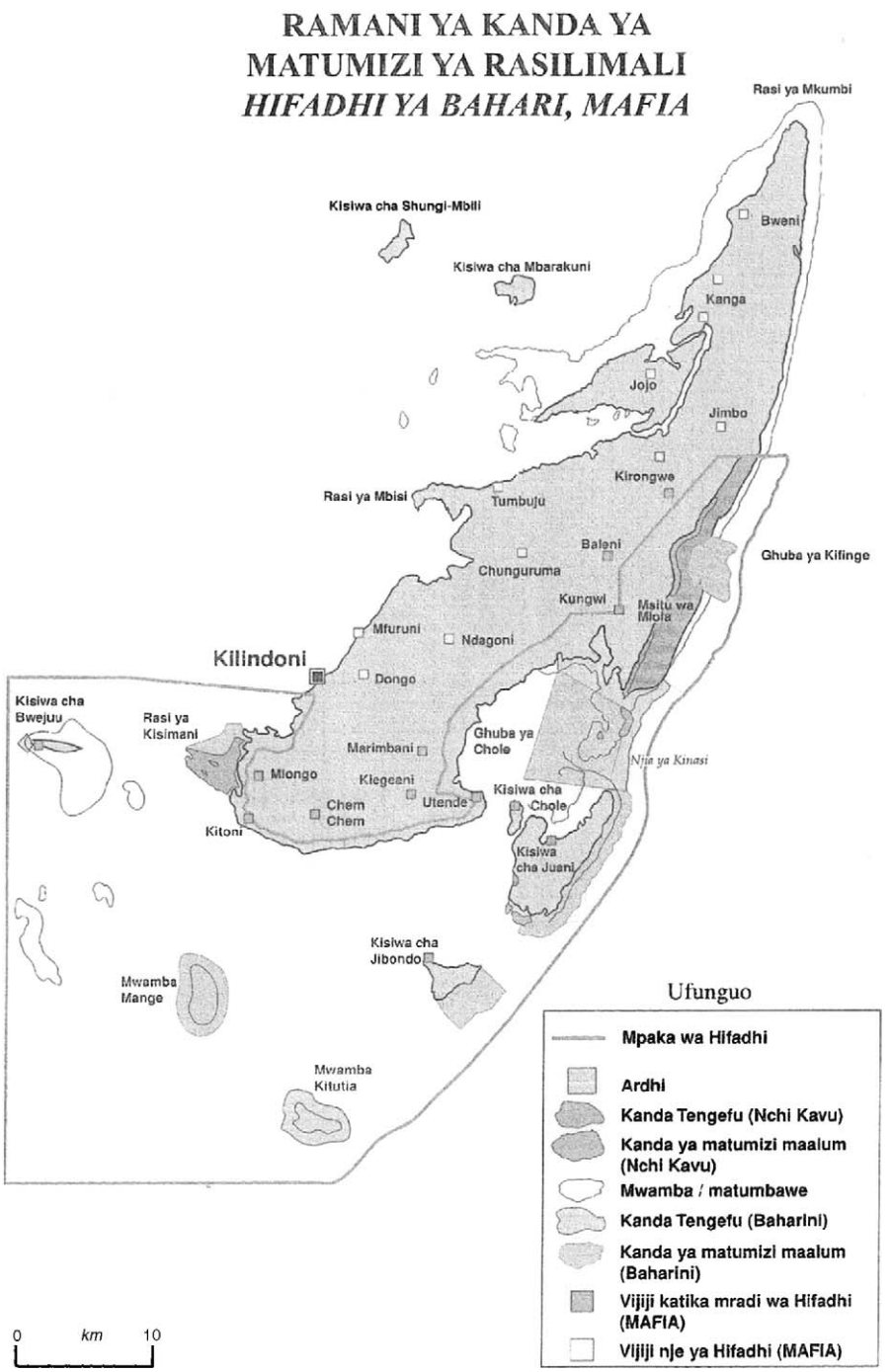

Fig. 1. Map of Mafia Island, United Republic of Tanzania indicating the villages in which cattle were sampled during two consecutive surveys in 2002.

of blood from injected mice) or from cattle found positive for non-pathogenic trypanosomes or with a PCV lower then 25, further samples were prepared for examination by polymerase chain reaction (PCR). Three heparinised micro-haematocrit capillary tubes were prepared, centrifuged using the MHCT method. The buffy-coat of these samples were placed on Whatmann filter paper no 3, stored between two Whatmann filter papers no. 4 and packed in plastic bags containing silica gel. On these samples, PCR was performed as described before (Geysen et al., 2002). From the blood collected in the plain vacutainer, serum was obtained and stored in Nalgene cryotubes for screening by Ab-ELISA test (S1 samples only) using a technique as described before (Mbwambo et al., 2000). Examination of smears and three months follow up on mice injection was carried out at the ADRI in Dar Es Salaam.

3. Results

3.1. Parasitological screening

Blood samples were taken from a total of 970 animals (i.e. 480 animals from S1 and 490 animals from S2). Table 2 gives details on the distribution of age, sex and breed of the cattle sampled. Using the DG-BC and confirmed by microscopic examination of the stained smears, evidence of the pathogenic trypanosomes of the *congolense* group (sub-genus Nannomonas) was found in two samples of Survey 1 (2/480) both in improved/cross cattle. Blood smear examination of samples of Survey 2 further revealed six samples (6/490) positive for pathogenic trypanosomes species of the sub-genus Nannomonas which was confirmed in one case by PCR and the species identified as *T. congolense* savannah type. Combining Surveys 1 and 2, a total of 8 (8/970) animals were found positive for pathogenic trypanosomes (0.8%) using microscopic techniques. Thirty animals (30/970) were found positive for the non-pathogenic *Trypanosoma theileri* (3.1%) and all these animals had a PCV above 28.

3.2. Mouse inoculation

For the two surveys combined a total of 67 blood samples of cattle, either positive on DG-BC or with a PCV lower than 25 and negative for DG-BC was injected into mice (0.5 ml) and checked over a period of 3 months post-inoculation. In S1, two mice (2/44) became positive for *T. congolense* on days 14 and 23, respectively and both had been injected with blood from animals found positive by DG-BC for *T. congolense*. In S2, one mouse (1/23) became positive for *T. congolense* on day 46 post-inoculation. The sample was taken from a 17-year-old Zebu cow, negative for trypanosomes by DG-BC technique but positive on blood smear. The remaining mice remained negative for the three months observation period.

Table 2
Numbers, age, sex and breed of cattle sampled during two surveys respectively in February 2002 (Survey 1) and August–September 2002 (Survey 2) on Mafia Island, United Republic of Tanzania

		Survey 1 (S1)		Survey 2 (S2)		S1 + S2	
		Numbers	%	Numbers	%	Numbers	%
Age	<2 years	130	27.1	132	26.9	262	27.0
	2–3 years	119	24.8	177	36.1	296	30.5
	4–6 years	92	19.2	89	18.2	181	18.7
	7–10 years	96	20.0	60	12.2	156	16.1
	>10 years	43	9.0	32	6.5	75	7.7
Sex	Female	373	77.7	382	78.0	755	77.8
	Male	107	22.3	108	22.0	215	22.2
Breed	Local	290	60.4	285	58.2	575	59.3
	Improved/cross	190	39.6	205	41.8	395	40.7
Total		480		490		970	100

Table 3
Comparing parasitological and PCR methods for positive cattle (including the cattle ID numbers) and location for cattle sampled during Survey 2 (September 2002), Mafia Island, United Republic of Tanzania

Region	Microscopic results		Results of PCR			
	T. congolense	*T. theileri*	*T. congolense*	*T. theileri*	*T. theileri*-like	negative
North		226	238[a]	238[a]		226, 236, 243, 256
Centre	332[b] 401[b], 412[b]	352[b], 399	421	299, 398, 404, 408, 426, 427	310, 313, 327, 343, 356, 357 390, 399	264, 333, 351
East-Centre	368[c], 458[d], 469[b]	435, 453, 458, 84, 85, 87	458	442, 453, 84, 85, 87, 103, 104	95, 97	435, 109
East		179, 189, 207		179, 189	207	186
South-east		58[a], 76		76	53, 82	
South		146, 156, 130		146, 151, 156, 130		159
City (Kilindoni)		8, 41, 42		8, 18, 36, 42	5, 25	19, 41
Total	6	20	3	25	15	13

[a] Mixed infection.
[b] Not checked by PCR.
[c] Positive by mouse inoculation for *T. congolense* by day 46 post-inoculation and blood smear, not checked by PCR.
[d] Trypanosomes of different sizes and shapes as well as akinetoplastic form observed and positive for *T. congolense* by PCR.

3.3. Ab-ELISA

The combined (not differentiated) prevalence of antibodies against *T. congolense* and *T. vivax*, using the *T. congolense* and *T. vivax* Antibody ELISA test, was 68/480 (14.2%) in S1. No ELISA tests were carried out on the samples of the second survey (S2).

3.4. PCR

Combining the two surveys, PCR-test was carried out on a total of 59 samples of which 6 were microscopically (DG-BC, blood smear or mouse inoculation) positive for pathogenic trypanosomes and a further 53 samples were either microscopically positive for non-pathogenic trypanosomes or of which the PCV was lower than 25. PCR showed six samples positive for pathogenic trypanosomes. For S1, PCR was carried out on 4 samples; the 2 samples which were found positive by DG-BCT for pathogenic trypanosomes and the 2 samples (out of 11 found), which were found positive for *T. theileri* and screened by PCR were confirmed as being *T. congolense* and *T. theileri*, respectively. For S2, PCR-test was carried out on 55 samples, either positive for trypanosomes or of animals with a low PCV. The test revealed 27 positive samples (of which one was a mixed infection); 3 samples were positive for *T. congolense* type savannah and 24 samples positive for the non-pathogenic *T. theileri.* The remaining 28 samples were negative for trypanosomes. Table 3 provides an overview on the comparison between parasitological screening and PCR results for Survey 2 on Mafia Island. From the *T. congolense* positive animals one was in a local breed aged over 15 years and the other animals were improved breeds and young animals (1–2 years).

4. Discussion

The results of the trypanosomosis surveys indicate that the prevalence of pathogenic trypanosomes on Mafia Island is low i.e. a prevalence of 0.8% (8/970) (using microscopical techniques) and 14.2% (68/480) (using Ab-ELISA technique), respectively. Although the presence of detected trypanosomal antibodies does not necessarily indicate a current infection, the combination of serological/parasitological and the PCR technique do support this low prevalence of trypanosomosis in cattle. Twelve out of the 13 PCR-trypanosome-negative cases had poor PCV values (20–26), yet no other blood parasites were detected.

The low prevalence of pathogenic trypanosomes is somewhat surprising in view of the island-wide distribution of the tsetse fly, *G. brevipalpis* being the only tsetse fly species so far encountered on the

island (IAEA, 2001). This low prevalence might be attributed to a possible combination of factors such as: (1) the frequent prophylactic treatment of the cattle with trypanocidal drugs, (2) a low feeding frequency of the tsetse fly *G. brevipalpis* on cattle or (3) the low vectorial capacity of *G. brevipalpis* for either *T. vivax* and/or *T. congolense.*

Regular prophylactic treatments with trypanocidal drugs will obviously mask the true epidemiological situation of the disease trypanosomosis. Unfortunately, no published data have been found indicating a possible number of drug treatments routinely administered to livestock on Mafia Island but it appeared that farmers treat their animals often themselves with drugs that are freely available on the market. More accurate data on the regular treatment of cattle would enable to determine a potential effect of these treatments on the dynamics of the disease.

There is a large population of *Potamochorerus larvatus* (bush pig) on Mafia Island and *Hippopotamus amphibius* is likewise present in a lake centrally situated at the island and it is known that *G. brevipalpis* feeds on these species which may act as reservoir of trypanosomes mainly *Trypanosoma simiae* (Kiragu, 1997). *G. brevipalpis* is also mainly known as a vector for *T. simiae* which is pathogenic for domestic pigs but not for wild pigs or cattle. For the two cases from S1, positive by DG-BC for pathogenic trypanosomes, the PCR test was deemed necessary to distinguish between the microscopically similar species *T. simiae* and *T. congolense*, which could both possibly have been present as indicated by field observations: Weitz (1963) classified *G. brevipalpis* with those tsetse species that feed mainly on mammals other than pigs or bovids. Studies in Uganda indicated that *G. brevipalpis* took most of its blood meals from bushpigs (45.5%) bovids (25.6%) and hippopotamus (28.5%) (Moloo et al., 1980). In a more recent study, ELISA techniques were used to identify the blood meals of *G. brevipalpis* and revealed that 78% of the blood meals originated from hippopotamus, whereas 8.6% were from wild Suidae and only 1.07% originated from cattle (Clausen et al., 1998). As was observed in The Gambia, *G. m. submorsitans* was feeding for 90% on the warthogs indicating their importance as maintenance hosts for the fly but not as a reservoir of pathogenic trypanosomes for livestock (Rawlings et al., 1993). Including blood meal analysis from *G. brevipalpis* in future studies will identify host-preferences and possibly confirm the low feeding preference of *G. brevipalpis* on cattle.

The widespread distribution of *G. brevipalpis* and the low prevalence of pathogenic trypanosomosis in livestock seems to indicate that *G. brevipalpis* is a poor vector for pathogenic trypanosomes on Mafia Island. The suitability of *G. brevipalpis* as an efficient vector for the pathogenic trypanosomes was clearly demonstrated during laboratory experiments by Moloo et al. (1988); very high mature infection rates for *T. vivax* but lower for *T. congolense* were obtained and the infection became better established in *G. brevipalpis* as compared to other *Glossina* spp. both for *T. vivax* and *T. congolense*. On the contrary, infection rates in the field are usually quite low as demonstrated in field studies in Kenya (Leak and Rowlands, 1997) and in Uganda (Harley, 1965). Both laboratory studies and field observations have indicated that *G. brevipalpis* shows markedly lower infection prevalence for pathogenic trypanosoma species as compared to species belonging to the *Morsitans* group (Harley, 1966; Moloo et al., 1998). Mihok et al. (1992) concluded that, although *G. brevipalpis* is usually present at lower densities as compared to other species, its significance as a vector should not be underestimated. Tsetse density alone does not necessarily indicate the trypanosome challenge and other parameters need to be taken into account when evaluating trypanosome challenge in a give area (Moloo et al., 1980).

In addition to the potential low 'vectorial capacity' of *G. brevipalpis* of Mafia Island, the virulence of the strains of the pathogenic trypanosomes seems also to be low since the two animals found positive for *T. congolense* savannah type had a PCV of 36 and 25, respectively and were clinically not suffering from any form of disease. The long incubation period following injection of blood of the two positive animals in mice might be an indication of lower pathogenicity of the infecting trypanosomes (for mice) however, the main reason is more likely the low number of parasites in the inoculum, given the negative DG-BC but positive blood smear. Wilson et al. (1972) observed that *G. brevipalpis* is capable of transmitting *T. congolense* and *T. vivax* but with lower infectivity; of trypanosomes of the *T. congolense* group recovered from the proboscis and salivary glands of *G. brevipalpis*, 24.2% infected mice and for the *T. vivax* group 17.3%

infected cattle. Compared to other *Glossina* species, all infections recovered from *G. brevipalpis* showed low infectivity and it was suggested that this might be due to differences in the gut of different *Glossina* species so that *T. congolense* can survive and develop better in the gut and hypopharynx of some flies as compared to other species (Moloo and Kutuza, 1988). Mihok et al. (1993) found that also host blood may influence the infection rates of trypanosomes in tsetse flies e.g. goat and pig blood facilitated infection in the tsetse flies whereas blood from cows and wildlife produced low infection rates.

There was a high percentage of cross-breeds in the samples as compared to local cattle during both surveys. Most of the crosses were found in the central part of the island and less in the North and South East of the island. At certain occasions apparently pure Holstein and Ayrshire cows were present and some farmers reported milk-yields up to 8 l/day. Often the cows were in the herd for over 10 years and had calved at regular intervals. Cross-breeds are in general much more susceptible to the effects of trypanosomes. This is again an indication that the *G. brevipalpis* seems to be a poor transmitter of the disease.

T. theileri, a cosmopolitan, non-pathogenic parasite of domestic cattle which does not undergo cyclical development in tsetse flies (Leak, 1998), was found in 3.1% of the samples. *T theileri* are transmitted by the omni-present Tabanidae but data on the densities of Tabanidae on Mafia Island are lacking. Parasitaemia is mostly low and difficult to detect on DG and a much higher percentages are found when blood culture is used to detect the carriers in a group of animals (Farrar and Kiel, 1990; Verloo et al., 2000).

It has been documented that *T. theileri* seem to become pathogenic when stress conditions arise (Ward et al., 1984; Hussain et al., 1985) or when infections with other pathogens are present e.g. East Coast Fever (ECF) (Carmichael, 1939; Townsend et al., 1982). It was noted that there were general complaints by farmers as East Coast fever (ECF) being a constraint to livestock. However, all animals found in this survey still had high PCV's. Clinical cases of *T. theileri* without clear evidence of an inter-current infection or immune-suppression are also reported (Doherty et al., 1993). Higher prevalence was found in beef cattle as compared to dairy cattle which related to the management and the resulting presence of the main vector the Tabanidae (Hussain et al., 1985; Farrar and Kiel, 1990).

There is evidence that *T. vivax* (Wells, 1972; Desquesnes and Dia, 2003a, 2004) and *T. congolense* (Desquesnes and Dia, 2003b), can be transmitted mechanically by biting flies such as Tabanidae and *Stomoxys*, however this is limited, exceptional and several conditions have to be fulfilled at the same time to reach a successful transmission. In regions where tsetse was eradicated and where biting flies were present, infection with *T. vivax* always disappeared so it is questioned if these biting flies alone can sustain the infection. Data from Zanzibar (Saleh et al., 1999) clearly indicate that even a very high *Stomoxys* population was not capable of sustaining the transmission of both *T. congolense* and *T. vivax* on Unguja Island, after *G. austeni* was eradicated. The low prevalence of *T. vivax* and *T. congolense* on Mafia seems to confirm this indication that mechanical transmission is not important from an epidemiological point of view.

The low prevalence of pathogenic trypanosomes on Mafia Island, as evidenced by the results of the surveys combined with general observations on the numbers and the health of improved dairy cattle present on the island, indicate that trypanosomosis is not the main disease constraint to livestock development, however, it remains a fact that pathogenic trypanosomes are present. The results of the survey also demonstrate the importance of collecting detailed information on tsetse densities and trypanosomosis prevalence in cattle before contemplating the development of a tsetse intervention strategy. The data indicate that control of trypanosomosis on Mafia Island could be done by the prophylactic and curative treatment of livestock with trypanocidal drugs. It would be useful to further investigate the link between the Glossina species present, the transmission risk and the trypanosomes found in the cattle including PCR test on all positive samples before embarking on an area-wide integrated campaign with a SIT component. Future surveys should therefore include collection of data on treatment of cattle with trypanocidal drugs, screening by PCR technique on all blood samples collected, either positive for trypanosomes or with PCV lower then 25 and blood meal analysis from *G. brevipalpis* in order to identify feeding host-preferences in addition to PCR-test in order to identify trypanosome infections in captured *G. brevipalpis*.

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