Monitoring the incidence of trypanosomosis in cattle during the release of sterilized tsetse flies on Unguja Island, Zanzibar

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Key words

Trypanosoma congolense - *Trypanosoma vivax* - Trypanosomosis - *Glossina austeni* - Morbidity - Zanzibar.

Summary

The incidence of trypanosomosis in sentinel cattle on Unguja Island, Zanzibar, was monitored every two to five months in 1994-97 to observe changes in disease transmission attributable logically to the application of insecticides, the release of sterilized tsetse flies (*Glossina austeni* Newstead) and the consequent decline and eradication of the wild tsetse population. Two parasitological techniques (microhematocrit centrifuge and buffy coat) were used to monitor the disease incidence caused by *Trypanosoma congolense* Broden and *T. vivax* Ziemann. *T. congolense* and *T. vivax* were detected in 1994 and 1995, but only *T. vivax* was detected thereafter. By 1997, the incidence of bovine trypanosomosis was only 0.1%. There was evidently no increase in disease incidence due to the release of sterilized isometamidium chloride-treated male tsetse flies.

■ INTRODUCTION

Trypanosomosis has been a major constraint to livestock production on Unguja Island, Zanzibar, United Republic of Tanzania. According to a 1993 livestock census, there were about 45,000 cattle on the island. *Glossina austeni* Newstead, the only tsetse fly species on the island, has been responsible for the cyclical transmission of animal trypanosomosis caused by *Trypanosoma congolense* Broden and *T. vivax* Ziemann. The disease was first diagnosed in Zanzibar in 1908. The first trypanosomosis survey, carried out between 1948 and 1951, revealed an overall infection rate of 17% caused predominantly by *T. congolense*, followed by *T. vivax* (16). During a survey of cattle conducted from October 1986 to July 1987 and based on the thick blood smear method, 49% of blood samples were found to be positive for trypanosomes, and 99% of positive cases included the presence of the parasite *T. congolense*, but *T. vivax* was encountered occasionally (5). Subsequently, attempts were made to control tsetse flies with insecticides. Pest and disease suppression was somewhat achieved, but the tsetse fly was not eradicated, especially in southern Unguja (1, 2, 32). A project was therefore initiated in 1994 to eradicate the tsetse fly by the sterile insect technique (SIT) (10). The project was completed successfully in 1997 (17, 30, 31, 36). During the SIT project the incidence of trypanosomosis in sentinel cattle was monitored to observe changes in disease transmission attributable logically to the application of insecticides, the release of sterilized tsetse flies and the consequent decline and eradication of the wild tsetse population.

There were two major tsetse fly SIT projects in the 1980's. Since the ultimate objective of vector eradication is cessation of trypanosome transmission and disease control, both projects monitored the disease as well as the fly population. In 1983-84, SIT was used to eradicate *Glossina palpalis gambiensis* and *G. tachinoides* in the Sideradougou pastoral zone (3500 km^2) in Burkina Faso (6, 7). Subsequent to tsetse eradication, a 1986-87 survey of trypanosomes in cattle in the control zone showed that *T. congolense* had disappeared, while a low level of *T. vivax* still remained (3, 6). Then, in 1985-87 *G. palpalis palpalis* was eradicated in an agropastoral area (1500 km^2) (BICOT project) in southern Plateau State, Nigeria (24). However, it was found that infections persisted in the project area's resident and sentinel cattle, possibly because of the presence of *G. tachinoides* or of the migratory activities of the cattle (12). Even though disease elimination seems probable following fly eradication, in these projects tsetse control or eradication did not guarantee the elimination of trypanosomosis in the control area.

This paper describes the results of monitoring the disease incidence during the tsetse SIT project in Zanzibar.

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■ MATERIALS AND METHODS

Study blocks and sentinel animals

Unguja Island was divided into 38 arbitrarily delineated blocks (figure 1) so that each block contained approximately 1000 cattle. The sentinel animal system was initiated in northern Unguja (blocks 1-29 and 38) early in 1994 and in southern Unguja (blocks 30-37) near the end of 1994. In each block, cattle (under two years old, preferably female and available for repeated blood samples) were selected as sentinel animals, and they were distributed in the herds of local cattle owners. In order to use sentinel animals that represented the block in which they were kept and their owners lived, the cattle were selected in proportion to the location and number of bovines resident within a block. During this study, sentinel cattle were not moved out of their designated blocks. Sentinel animals were identified by village, owner, sex and external physical features such as color markings.

It was planned that blood samples would be taken repeatedly from the same sentinel animals throughout the project, but not all sentinel animals were available on every sampling date, so some data could not be recorded. In northern Unguja the average number of sentinel animals sampled per block ranged from 12 to 33, depending on the sampling date. In the north, it was not possible on each sampling date to get a consistently high number of animals due to the reluctance of some cattle owners to permit that blood samples be taken from their animals. However, in the south, the owners were more cooperative, and the average number of animals sampled per block was higher, ranging from 29 to 59, depending on the sampling date.

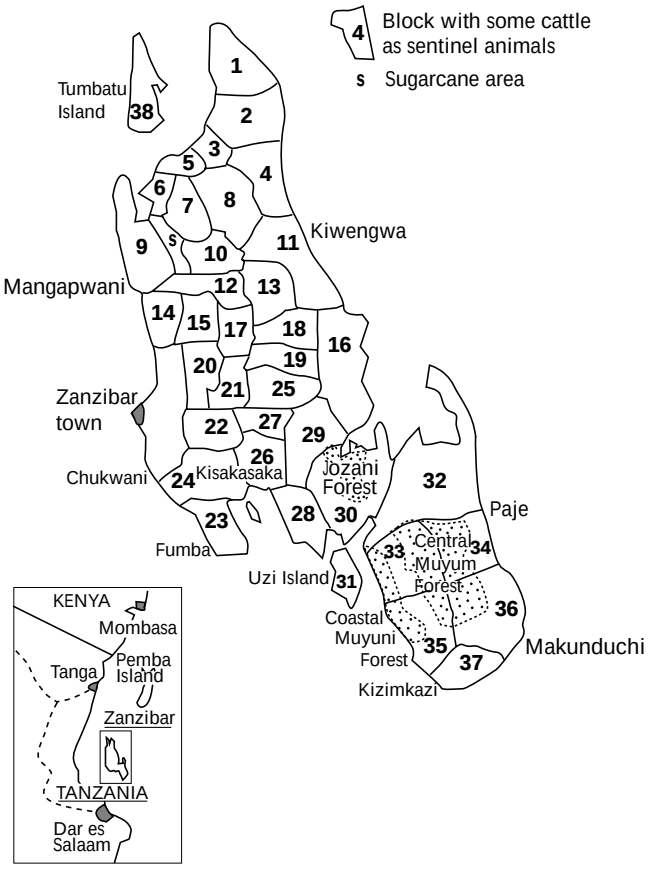

Figure 1: *Map of Unguja Island showing the location of 38 blocks, each block containing sentinel cattle.*

The sentinel animals were initially treated intramuscularly with diminazene aceturate (BERENIL®, Hoechst AG, Frankfurt am Main, Germany) at 7 mg/kg. Two months after treatment, the regular blood-sampling program was launched to test for trypanosomes. To avoid misrepresentation, veterinarians on the island were instructed not to treat sentinel animals with trypanocidal drugs, and no unauthorized treatment was made. If a sentinel animal died or was slaughtered during the project, another suitable animal replaced it after the same treatment procedure had been followed.

Blood samples

Blood samples from sentinel cattle were taken every 2-5 months until near the end of 1997 (animals were sampled 13 times in northern Unguja, and 12 times in southern Unguja). Each animal which tested positive for trypanosomes was treated with diminazene aceturate at 7 mg/kg one or two days later (27), but beginning in late 1996 all sentinel animals were treated routinely with diminazene aceturate immediately after a blood sample was taken.

As each animal was surveyed, 5 ml of blood were collected from the jugular vein into a plain vacutainer. About 1 ml of the blood was poured into a 2-ml vial containing ethylenediaminetetraacetic acid (EDTA) to prevent blood from clotting, it was then put into a cool box until arrival at the laboratory a few hours later. In the laboratory, a microhematocrit reader was used for determination of the packed red cell volume (PCV) (27). Also, the microhematocrit centrifuge technique (MHCT) (39) and the buffy coat technique (BCT) (22, 28) were performed immediately on all samples. MHCT and BCT were repeated on all samples that had a PCV below 25% since the anemic animal might have trypanosomosis. In the few instances of mixed infection when an animal was positive for both *T. congolense* and *T. vivax*, the animal was regarded as half positive for one species and half positive for the other so as not to exaggerate the overall disease incidence.

Data handling

The software EPI-5 or EPI-6 (8) was used to record in a computer the data from the blood samples. All data (block number, name of village and cattle owner, description and age of individual cattle, date and time of each blood sample, results of PCV, MHCT and BCT tests) were entered. Assessment of trypanosome transmission was based on a change from negative to positive status in a sentinel animal.

Fly and disease control activities

In 1994 and 1995, to assist in suppressing the wild fly population, deltamethrin, as a pour-on formulation (SPOT-ON®, Pitman-Moore, Middlesex, UK) or as a cattle dip (DECATIX®, Pitman-Moore, Middlesex, UK), was applied about every other month to as many cattle and goats on the island as practically possible. As an incentive for the owners of sentinel cattle to cooperate with the blood-sampling program, sentinel cattle were included in these treatments. The owners wanted deltamethrin to be used because of the benefit of tick control. However, starting in 1996 when the wild fly population was at a low level, only sentinel cattle were treated with the pour-on formulation in order to maintain the cooperation of farmers owning sentinel cattle.

The aerial release of sterilized male tsetse flies commenced in August 1994 in southern Unguja prior to the first blood sample in the south (collected in January 1995), and the release started in northern Unguja in August 1996 following the tenth blood sample in the north (31). Release of sterilized males continued until the end of 1997 (36).

An enigmatic disease (*T. vivax*) outbreak developed in southern Unguja in 1995. To determine the cause of this outbreak, and to reduce the disease problem and prevent its reoccurrence, several actions were taken.

1. During the period of June to August 1995, 77 live sterilized male flies were collected from sticky traps in the Muyuni regions of southern Unguja (figure 1) (36) and were dissected on the same day they were collected. The proboscis (labrum and hypopharynx), gut and salivary glands were examined for pathogenic trypanosomes (29). In addition, during the same period, 62 live sterilized females were collected and dissected for trypanosomes.

2. Early in the project, when insufficient sterile male flies were available for release, the possibility that sterilized female flies could be a "sperm sink" for wild males, and thus contribute to fly control, was tested. Sterilized females were released experimentally from October 1994 to August 1995 in tsetse-unsuitable habitats in southern Unguja (extreme southern and eastern areas where sterilized males had not yet been released (37), to minimize the risk of disease transmission). However, when the *T. vivax* disease outbreak occurred in southern Unguja in August 1995, sterilized female releases were terminated, just in case the female flies were contributing to the outbreak.

3. Even though it was unlikely that the released sterilized male flies would transmit trypanosomosis, as a precaution starting in September 1995, all male tsetse flies destined for release were fed twice prior to release on blood mixed with isometamidium chloride (12.5 µg/ml blood) (SAMORIN®, RMB Animal Health, Dagenham, UK) (20, 21).

4. Also, in September 1995, all sentinel cattle in southern Unguja were treated with diminazene aceturate, and all other available cattle (about 3400) in southern Unguja were treated with isometamidium chloride. In addition, a pour-on or cattle-dip application of deltamethrin was performed on all available cattle in the area.

RESULTS

In northern Unguja the disease incidence was very low, always below 1% (figure 2). *T. congolense* was detected in only four animals out of 3152 sampled (0.13%), and only in 1994. In 1995-97 one animal positive for *T. vivax* was found in seven different sample periods, including the last period, but it was never the same animal. The positive case in August 1997 was an animal that, due to its unavailability in April, had not been sampled for nine months.

In southern Unguja the disease incidence was considerably higher (figure 3). However, *T. congolense* was detected in relatively few cattle and not after 1995. Following the trypanocidal treatment in September 1995 of virtually all cattle in southern Unguja because of the *T. vivax* outbreak, the incidence of *T. vivax* greatly declined. In 1996, only ten cattle out of 1135 sampled (0.88%) were positive for *T. vivax*, and in 1997 only one out of 520 sampled (0.19%). The overall disease incidence in the sentinel cattle in 1997 was only 0.1%.

In mid 1995, 77 sterilized male flies trapped in the Muyuni villages (southern Unguja) were dissected for trypanosomes, but no trypanosome was found. Only one out of 62 trapped sterilized females showed an infection with *T. vivax*.

DISCUSSION

Due to the absence of tsetse flies and the unlikelihood of tsetse reinvasion, it is not expected that transmission of *T. congolense* and regular transmission of *T. vivax* will occur in the future. Even if there is mechanical transmission by other biting flies (4, 14, 18, 19, 34, 38)—in South America tabanids can transmit *T. vivax* (13, 25, 26)—, a rather controversial subject at the epidemiological level in Africa (15, 35), it is concluded that trypanosomosis is probably not sustainable on Unguja Island (30). Analysis of regular blood samples taken in 1998 and 1999 using MHCT and BCT indicated that no trypanosomosis transmission occurred following the 1997 completion of the tsetse fly eradication program, even in the presence of *Stomoxys* spp. (primarily *S. niger niger* Macquart) (30). This is an important finding, indicating that tsetse eradication using SIT has resulted in sustained disease control!

The practical necessity to apply deltamethrin to sentinel cattle in order to obtain farmers' cooperation, though undesirable from a scientific point of view, does not decrease the validity of the conclusion that the disease incidence decreased over time. Deltamethrin applications to sentinel cattle were made throughout the study period, so any effect on tsetse flies and disease transmission would have been uniform throughout.

It is recognized that MHCT and BCT are not sensitive enough techniques to detect the low numbers of parasites, due to fluctuating parasitemia, characteristic during the chronic stage of the disease (23). Nevertheless, the trends in the data, which were obtained in a consistent manner, support the conclusions made.

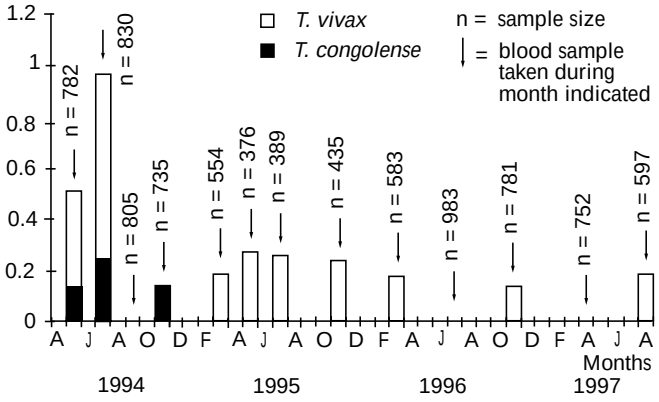

Figure 2: *Incidence (%) of trypanosomosis in sentinel cattle in northern Unguja during 1994-1997 according to the microhematocrit centrifuge and buffy coat techniques.*

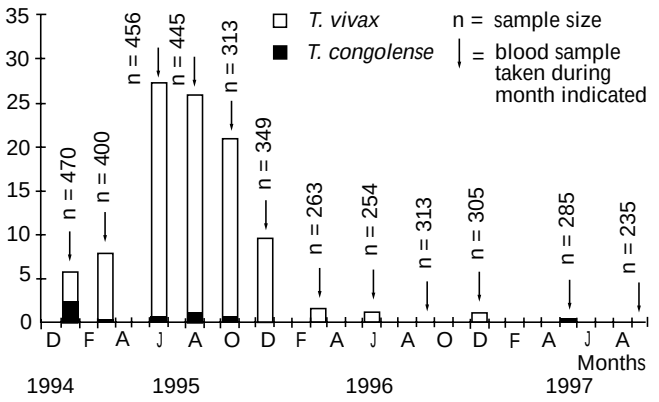

Figure 3: *Incidence (%) of trypanosomosis in sentinel cattle in southern Unguja during 1995-1997 according to the microhematocrit centrifuge and buffy coat techniques.*

During the early stages of the project, a serological technique, the antigen-detection ELISA, was used as an additional parameter for assessing disease transmission. Preliminary results using this ELISA technique were reported earlier (27), but recently they were found to be unreliable and difficult to interpret (9, 11), and the use of this technique was discontinued in 1997. Future monitoring of the disease situation with more sensitive techniques, such as the polymerase chain reaction (PCR) (33), would assist in evaluating the status of trypanosomosis.

The 1995 *T. vivax* outbreak in southern but not in northern Unguja is an enigma. Since the wild fly density was quite low in the southern portion of the island, it appears that the experimental release of sterilized female flies (October 1994-August 1995) might have contributed to disease transmission. However, almost none of the trapped females was found to be infected, and the high disease incidence in southern Unguja was more widespread than the small area in which females were released. The possibility that the outbreak was related to an environmental factor that caused stress in the cattle (4, 35) cannot be assessed properly since long-term data on disease prevalence prior to the SIT project were not collected.

■ CONCLUSION

The incidence of trypanosomosis on Unguja Island was quite low in 1994, probably due to the intensive insecticidal treatments to control the tsetse fly vectors in previous years, especially in northern Unguja. The incidence of *T. congolense* decreased to a negligible level by 1995, and that of *T. vivax* by 1996, the logical result of a declining wild tsetse fly population subjected to insecticide followed by SIT.

There was no direct evidence from fly dissection that released sterilized male tsetse flies transmitted trypanosomosis. Also, there was evidently no increase in disease incidence due to the release of these flies.

Acknowledgments

We express our gratitude to H.U. Feldmann and M.J.B. Vreysen for their constructive criticism of the manuscript, and to A.G. Parker and H.U. Feldmann for preparing the map of Unguja Island. We thank M.R. Mzee, A.M. Idrissa, S.S. Bedwi, S.M. Haji and A.M. Ngwali for their dedicated work in collecting and analyzing blood samples. We are grateful to A.R. Msangi, A.G. Parker and the scientific officers of the Tsetse and Trypanosomosis Research Institute, Tanga, for their advice in the course of this project. The cooperation of the many cattle owners involved is highly appreciated.

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Reçu le 26.07.1999, accepté le 17.04.2001

Résumé

Dyck V.A., Pan H., Kassim S.S., Suleiman F.W., Mussa W.A., Saleh K.M., Juma K.G., Mkonyi P.A., Holland W.G., van der Eerden B.J.M., Dwinger R.H. Surveillance de l'incidence de la trypanosomose chez les bovins pendant les lâchers de mouches tsé-tsé stérilisées sur l'île d'Unguja, à Zanzibar

L'incidence de la trypanosomose chez des bovins sentinelles sur l'île d'Unguja à Zanzibar a été contrôlée tous les deux à cinq mois entre 1994 et 1997 afin d'observer les changements dans la transmission de la maladie, logiquement attribuables à l'application d'insecticides, aux lâchers de mouches tsé-tsé stérilisées (*Glossina austeni* Newstead) et à la diminution consécutive, jusqu'à l'éradication, de la population naturelle de glossines. Deux techniques parasitologiques (centrifugation microhématocrite et examen du *buffy coat*) ont été utilisées pour surveiller l'incidence de la maladie due à *Trypanosoma congolense* Broden et à *T. vivax* Ziemann. *T. congolense* et *T. vivax* ont été détectés en 1994 et en 1995 mais, par la suite, seul *T. vivax* a été observé. En 1997, l'incidence de la trypanosomose bovine n'était plus que de 0,1 p. 100. Elle n'a manifestement pas augmenté après les lâchers de mouches tsé-tsé mâles stérilisées et traitées au chlorure d'isométamidium.

Mots-clés : *Trypanosoma congolense* - *Trypanosoma vivax* - Trypanosomose - *Glossina austeni* - Morbidité - Zanzibar.

Resumen

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La incidencia de la tripanosomosis en ganado centinela en la Isla de Unguja, Zanzíbar, fue seguida cada dos a cinco meses, entre 1994-97 y esto con el fin de observar los cambios en la transmisión de la enfermedad, atribuibles de manera lógica a la aplicación de insecticidas, a la liberación de moscas tsé-tsé (*Glossina austeni* Newstead) esterilizadas y las consecuentes disminución y erradicación de la población de tsé-tsé silvestres. Se utilizaron dos técnicas parasitológicas distintas (la centrifugación por microhematocrito y la técnica de *Buffy coat*), con el fin de seguir la incidencia de la enfermedad causadapor *Trypanosoma congolense* Broden y *T. vivax* Ziemann. *T. congolense* y *T. vivax* fueron detectados en 1994 y 1995, pero únicamente *T. vivax* fue detectado ulteriormente. Hacia 1997, la incidencia de la tripanosomosis bovina fue de solo 0,1 %. No hubo un aumento evidente en la incidencia de la enfermedad debido a la liberación de moscas tsé-tsé macho esterilizadas mediante un tratamiento de clorídeo de isometamidio.

Palabras clave: *Trypanosoma congolense* - *Trypanosoma vivax* - Tripanosomosis - *Glossina austeni* - Morbosidad - Zanzíbar.