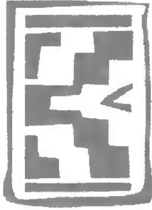

The parasitological and serological prevalence of tsetse-transmitted bovine trypanosomosis in the Eastern Caprivi (Caprivi District, Namibia)

P. VAN DEN BOSSCHE[1], D. MUDENGE[1], J. MUBANGA[2] and A. NORVAL[3]

ABSTRACT

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Between August 1995 and June 1997 a survey to determine the distribution of tsetse-transmitted trypanosomosis was conducted in the Eastern Caprivi (Caprivi District, Namibia). A total of 1 481 adult cattle was examined at 33 sampling sites. Direct parasitological diagnostic tests were used and eluted blood spots were screened for the presence of anti-trypanosomal antibodies.

Tsetse-transmitted trypanosomal infections were detected in 66 animals (4,5 %) from 14 different locations. The parasitological and serological prevalence of trypanosomosis was highest in the Mamili area. Trypanosomosis was virtually absent in the Linyanti/Chobe area and the target barrier along the Kwando River had significantly reduced the prevalence of trypanosomosis in cattle grazing to the east of it. This suggests that anti-trypanosomal antibody prevalence data can be used to evaluate and monitor the effectiveness of tsetse control measures. Survey results suggest that in the Katima Mulilo area, trypanosomal infections were being acquired when cattle grazed along the Zambezi River. Moreover, survey results indicate that tsetse have not been able to establish themselves in the Katima Mulilo area.

The parasitological prevalence in a herd and the respective prevalence of anti-trypanosomal antibodies was significantly correlated to the percentage of anaemic animals in that herd. Furthermore, the parasitological prevalence in a herd was positively correlated with the prevalence of anti-trypanosomal antibodies of that herd. It is concluded that the prevalence of anti-trypanosomal antibodies in a herd can be used as an additional indicator of the extent of infection in that particular herd.

Keywords: Caprivi, nagana, Namibia, prevalence, trypanosomosis, tsetse

INTRODUCTION

In Namibia, tsetse-transmitted trypanosomosis or "nagana" is restricted to the Caprivi District. The distribution of tsetse (*Glossina morsitans centralis*) is confined for the greater part to the Linyanti-Mashi-Kwando drainage. The main foci are located along the Kwando River between Kongola and the Angolan and Zambian borders in the north and around Lupala and Nkasa "islands" in the south (Bingham, De Rooij, Flanagan *et al.* 1995).

Between 1964 and 1994, human and animal trypanosomosis were controlled by ground-spraying operations along the eastern and western banks of the Kwando River (Bingham *et al.* 1995). In 1994, a 5 km-wide, odour-baited, insecticide-treated, target barrier (Hargrove 1993) was constructed along the western side of the Kwando River starting near the Botswana border and extending to the Angola border. In the same year, a similar target barrier was constructed along the northern edge of the Mamili National Park.

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In 1984, an outbreak of trypanosomosis in cattle was reported in the Katima Mulilo area. The presence of nagana in this area is of great concern especially because of the possible spread of tsetse southwards across the Caprivi strip to the Chobe River and, thence into Botswana. To establish the current distribution of tsetse-transmitted trypanosomosis in the Eastern Caprivi and to determine its spread in the Katima Mulilo area, a survey was conducted. Use was made of both parasitological and serological methods (antibody-detection). The value of these survey methods in establishing the distribution of tsetse-transmitted trypanosomosis is discussed in the light of the results obtained from the survey.

MATERIAL AND METHODS

Sampling area

The Eastern Caprivi lies to the east of the Kwando River (Fig. 1) in the Caprivi District (Namibia). It is more than 11 600 km^2 in extent and has a population of over 122 000 head of cattle. The only tsetse species occurring is *G. morsitans centralis*.

Between August 1995 and June 1997, a survey of bovine trypanosomosis was conducted at 33 sampling sites (Fig. 1).

To facilitate the interpretation of the survey results, the sampling area was subdivided into survey areas. Sampling sites were categorized according to the grazing areas of the cattle and allocated to one of the survey areas. Four survey areas were identified (Table 1):

- "Katima Mulilo area" (cattle grazing along the Zambezi and immediately south of Katima Mulilo)
- "Kwando area" (cattle grazing along the Kwando River, north of Ngonga)
- "Mamili area" (cattle grazing along the Kwando/ Mashi River north of the Mamili National Park)
- "Linyanti/Chobe area" (cattle grazing along the Linyanti and Chobe Rivers)

Odour-baited target barriers were in place west of the Kwando survey area and south of the Mamili survey area (Fig. 1). Trypanocides, mainly diminazene aceturate (Berenil®, Hoechst), were used frequently in cattle herds in the Mamili survey area.

Sampling size

A total of 1 481 adult cattle were examined. A cross-sectional sampling method was applied. Sample sizes were calculated according to Cannon & Roe (1982). They depended on the total cattle population at a particular sampling site but never exceeded 60 head of cattle at a single sampling site. This maximum sample size allows the detection of at least one positive case if trypanosomal infections are present at a prevalence level of 5 %—this provides a confidence level of 95 %.

TABLE 1 Survey areas and sampling sites in Eastern Caprivi

Survey area	Sampling site	Survey area	Sampling site
Katima Mulilo	Fooma	Linyanti/ Chobe	Sangwali
	Kalumba		Malinda
	Mpacha		Samutetesi
	Bito		Mbilanje
	Mubiza		Mrunga
	Sifuha		Kapani
	Bukalo		Chinchimani
	Kwena		Ibbu
	Masokotwani		Mukanwa
	Iseke		Masikili
	Silumbi	Mamili	Lianshulu
Kwando	Izwilii		Saujuo
	Kalubi		Nongozi
	Kongola		Mbambazi
	Ngonga		Lizauli
	Singalamwe		Samudondo
			Malengalenga

Sampling method

Direct parasitological diagnostic tests (Paris, Murray & Mcodimba 1982) were used. Blood was collected from an ear vein into heparinized microhaematocrit centrifuge capillary tubes and onto glass slides, as thick and thin blood films. The capillary tubes were sealed with "Cristaseal" (Hawksley) and centrifuged immediately in a microhaematocrit centrifuge for 5 min at 9000 rpm. After centrifugation, the packed cell volume (PCV) was determined. The buffy coat and the uppermost layer of red blood cells were extruded onto a microscope slide and examined for the presence of motile trypanosomes. Samples were examined with a phase-contrast microscope with a x 40 objective lens. Giemsa-stained thick and thin blood smears were examined under a x 100, oil immersion objective lens.

From each animal, blood contained in one heparinized microhaematocrit centrifuge capillary tube was extruded onto a filter paper (Whatman n° 4, Whatman®). Four different blood samples (blood spots) were collected on each filter paper. All filter papers were labelled indicating the date of collection, place of collection and the sample number. Samples were air dried out of direct sunlight and placed in a plastic bag with self-indicating silica gel desiccant. The bag was sealed and kept as cool as possible until the specimens were refrigerated or frozen. Eluted blood spots were screened for the presence of anti-trypanosomal antibodies using an indirect anti-trypanosomal antibody-detection Enzyme-Linked Immunosorbant Assay (ELISA) (Luckins 1977; Hopkins, Chitambo, Machila *et al.* 1998). Use was made of a *Trypanosoma congolense* (IL 3000) invariable antigen batch prepared by the Parasitology Laboratory of the

FIG. 1 Map of the survey area and location of sampling sites

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A rigorous system of quality assurance was adopted. Hence, each ELISA-microplate was run with strong positive, weak positive and negative reference sera which were required to comply with pre-set values. The Optical Density (OD) of each ELISA-sample tested was expressed as a percentage (Percentage Positivity, PP) of the strong positive reference standard (Wright, Nilsson, Van Rooij *et al.* 1993). A cut-off of 28 % positivity was used. At this cut-off the assay had a sensitivity of 88,5 % and a specificity of 99 %. The true seroprevalence was calculated using these sensitivity and specificity values (Thrusfield 1986).

RESULTS

Parasitological prevalence

A total of 1 481 samples was examined. Tsetse-transmitted trypanosomes were detected in 66 animals

TABLE 2 Sample size, number of animals with trypanosomal infections and mean PCV (± s.e.) of herds sampled at various sampling sites in the different survey areas

Survey area	Sample site	Sample size	Trypanosomal infections				Mean PCV (± s.e.)
			T. vivax	*T. congolense*	*T. brucei*	Mixed (Tc/Tv)	
Katima Mulilo	Mpacha	55	0	0	0	0	33,4 ± 0,5
	Bito	28	1	0	0	0	35,6 ± 0,4
	Kalumba	10	0	0	0	0	37,2 ± 1,1
	Kwena	30	0	0	0	0	30,5 ± 0,8
	Masokotwani	37	0	0	0	0	33,9 ± 0,5
	Mubiza	40	0	0	0	0	36,1 ± 0,7
	Fooma	42	0	1	0	0	31,2 ± 0,8
	Bukalo	34	0	0	0	0	31,7 ± 0,7
	Iseke	30	0	0	0	0	35,2 ± 0,7
	Sifuha	50	0	0	0	0	32,8 ± 0,6
	Silumbi	10	0	0	0	0	–
Kwando	Kalubi	50	0	3	0	0	32,3 ± 0,5
	Kongola	60	0	1	0	0	30,7 ± 0,5
	Izwilii	60	0	0	0	0	30,8 ± 0,7
	Singalamwe	60	0	2	0	0	31,6 ± 0,5
	Ngonga	60	0	0	0	0	32,5 ± 0,5
Mamili	Mbambazi	60	4	0	0	0	29,9 ± 0,7
	Samudondo	60	3	0	0	0	31,6 ± 0,7
	Lianshulu	60	7	0	0	0	24,4 ± 0,8
	Saujuo	33	12	0	0	0	27,0 ± 1,0
	Nongozi	60	3	0	0	0	28,3 ± 0,6
	Lizauli	60	21	2	0	0	30,0 ± 0,9
Linyanti/Chobe	Sangwali	60	3	0	0	0	31,1 ± 0,7
	Malinda	60	0	0	0	0	33,8 ± 0,6
	Malengalenga	60	0	0	0	1	29,8 ± 0,6
	Samutetesi	60	0	0	0	0	34,5 ± 0,6
	Mbilanje	60	0	0	0	0	31,4 ± 0,7
	Kapani	40	0	0	0	0	33,4 ± 0,4
	Mrunga	60	0	2	0	0	31,3 ± 0,4
	Chinchimani	20	0	0	0	0	34,9 ± 0,8
	Mukanwa	25	0	0	0	0	34,5 ± 0,9
	Ibbu	33	0	0	0	0	33,7 ± 0,7
	Masikili	14	0	0	0	0	–

TABLE 3 Number of samples, mean serological true prevalence, parasitological prevalence of trypanosomosis and mean packed cell volume of herds sampled in each of the survey areas

Survey area	Number of samples	Mean parasitological prevalence (± s.e.)	Number of samples	Mean serological true prevalence (± s.e.)	Number of samples	Mean PCV (± s.e.)
Katima Mulilo	397	1,2 ± 0,1	236	13,2 ± 1,3	422	33,6 ± 0,2
Kwando	290	2,2 ± 0,1	277	17,3 ± 1,4	288	31,2 ± 0,2
Mamili	360	11,4 ± 0,1	138	32,9 ± 0,6	272	28,0 ± 0,4
Linyanti/Chobe	432	0,8 ± 0,1	338	3,4 ± 0,3	398	32,2 ± 0,2

(4,5 %) sampled at 14 of the 33 sampling sites (Table 2).

All infections were detected on buffy coat and confirmed on thick and thin smears. The parasitological prevalence of *Trypanosoma vivax, T. congolense* and mixed (*T. congolense* and *T. vivax*) infections was 81,8 %, 16,7 % and 1,5 %, respectively. Overall parasitological prevalence was highest in the Mamili survey area. The proportion of *T. vivax* infections varied significantly between the survey areas. *T. vivax* infections were dominant in the Mamili area (95,9 %) whereas all trypanosomal infections detected in cattle grazing in the Kwando area were *T. congolense*.

The mean parasitological prevalence was low in the Katima Mulilo, Kwando and Linyanti/Chobe survey area (Table 3). A significantly higher mean parasitological prevalence was found in the Mamili survey area (Table 3).

Packed cell volume (PCV)

The average PCVs (Table 3) and PCV-profiles (Fig. 2) were significantly different between all survey areas ($P < 0,001$; t-test).

The percentage of anaemic animals (PCV < 25 %) at a sampling site and the parasitological prevalence of trypanosomal infections at the same sampling site were significantly correlated ($r = 0,71$; $P < 0,001$).

Serological prevalence of trypanosomal antibodies

A total of 1 196 blood spots were screened for trypanosomal antibodies (Table 4). Only 115 samples (9,6 %) were serologically positive ($P \geq 28$ %).

Trypanosomal antibodies were detected in herds sampled at 18 out of the total of 30 sample sites where blood spots were collected (Table 4). The serological true prevalence varied considerably between locations (Table 4). Cattle at three sampling sites (10 %), where trypanosomes were detected using parasitological methods, had no anti-trypanosomal antibodies. A significant correlation ($r = 0,58$; $P < 0,01$) was found between the parasitological and serological true prevalence of trypanosomosis at the various sampling sites.

Trypanosomal antibody titres were found in cattle from nine sampling sites where animals were parasitologically negative. Average serological true prevalence in the parasitologically negative herds was 7,9 % compared to 29,4 % in herds that were parasitologically positive. The mean serological true prev-

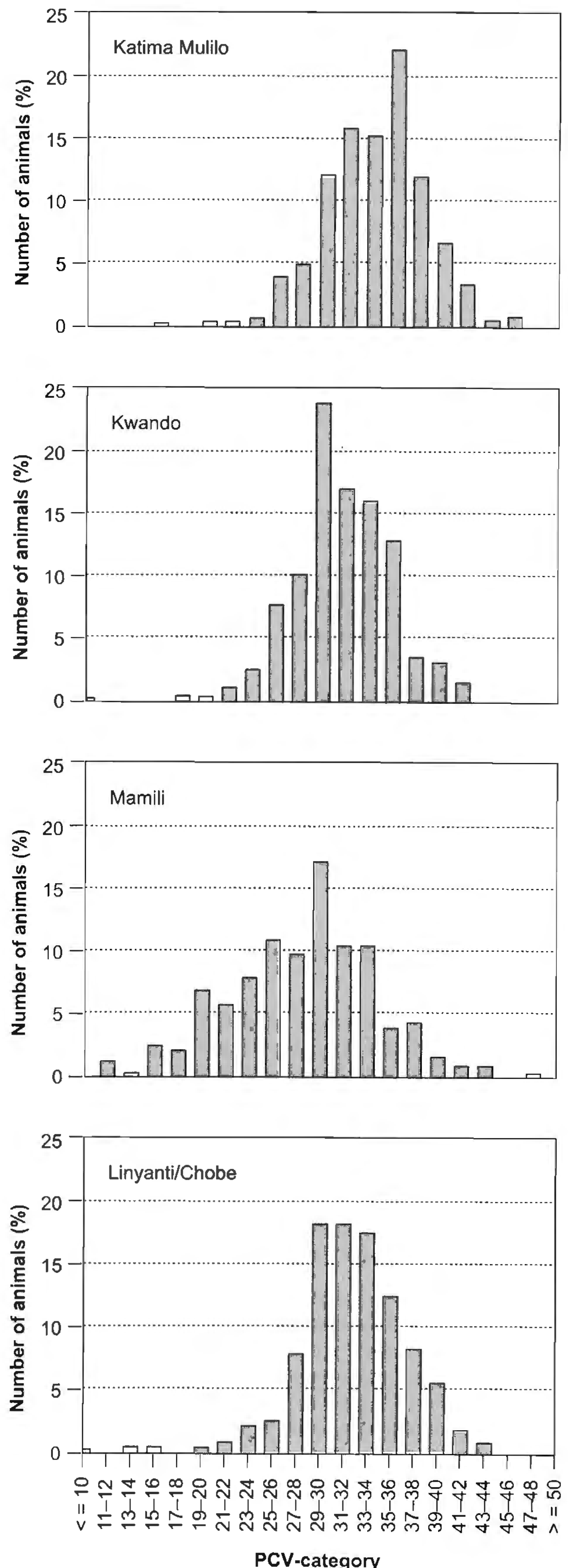

FIG. 2 Comparison of PCV-profiles of herds sampled in the Katima Mulilo, Kwando, Mamili and Linyanti/Chobe survey areas

TABLE 4 Sample size, number of positives, average percentage positivity (± s.e.) and serological prevalence of samples collected at various sampling sites in the different survey areas

Survey area	Sample site	Sample size	Number serological positive	Percentage positivity (± s.e.)	Serological prevalence (%)	
					Apparent	True
Katima Mulilo	Mpacha	49	14	21,3 ± 1,3	28,6	32,3
	Bito	27	0	16,5 ± 0,7	0	0
	Kalumba	10	0	16,6 ± 1,5	0	0
	Kwena	30	1	18,4 ± 0,8	3,3	3,7
	Masokotwani	47	0	16,6 ± 0,5	0	0
	Mubiza	39	2	16,4 ± 0,9	3,3	3,7
	Fooma	40	2	28,6 ± 1,9	50,0	56,5
	Bukalo	31	2	18,6 ± 0,7	6,5	7,3
	Iseke	32	0	18,7 ± 0,7	0	0
	Sifuha	46	1	17,9 ± 0,	2,2	2,5
	Silumbi	9	0	15,2 ± 1,8	0	0
Kwando	Kalubi	59	0	15,5 ± 2,0	6,7	7,8
	Kongola	57	4	21,5 ± 1,1	7,0	8,3
	Izwilii	59	0	15,5 ± 0,5	0	0
	Singalamwe	47	7	20,7 ± 1,2	14,9	17,5
	Ngonga	50	5	15,4 ± 1,1	10,0	11,8
Mamili	Mbambazi	53	18	22,2 ± 1,7	34,0	40,0
	Samudono	59	0	13,3 ± 0,7	0	0
	Lizauli	52	26	27,7 ± 1,7	50,0	58,8
	Lianshulu	52	15	24,5 ± 1,9	28,9	33,9
	Saujuo	33	7	21,2 ± 2,6	21,2	25,0
Linyanti/ Chobe	Sangwali	57	8	15,7 ± 1,5	14,0	16,5
	Samutetesi	54	1	12,1 ± 0,5	1,9	2,2
	Mbilanje	57	0	13,5 ± 0,5	0	0
	Kapani	37	0	8,4 ± 0,8	0	0
	Mrunga	57	0	14,6 ± 0,5	0	0
	Chinchimani	20	0	15,6 ± 0,7	0	0
	Mukanwa	23	1	17,9 ± 0,8	4,3	4,8
	Ibbu	33	1	20,2 ± 0,6	3,0	3,3
	Masikili	13	0	19,2 ± 0,9	0	0

alence in each of the survey areas is summarized in Table 3.

The percentage of anaemic (PCV < 25 %) animals at a sampling site was significantly correlated (r = 0,59; P < 0,01) with the serological true prevalence at that site.

DISCUSSION and CONCLUSION

Parasitological and serological prevalence of trypanosomosis in the Eastern Caprivi

According to the parasitological and serological prevalences, tsetse-transmitted trypanosomal infections in the Eastern Caprivi are confined to the Kwando River drainage and the close vicinity of Katima Mulilo. The Kwando River infestation complies with the scanty information on the historical distribution of tsetse in the Eastern Caprivi (Bingham *et al.* 1995). However, the Kwando River target barrier seems to have significantly reduced the spread of tsetse. The effectiveness of the target barrier in reducing tsetse challenge is clearly reflected in the low parasitological prevalence of trypanosomosis and the low prevalence of anti-trypanosomal antibodies in cattle sampled in the Kwando survey area.

South of the barrier, the parasitological prevalence of trypanosomosis was unexpectedly high. This is explained by the recent capture of tsetse at Lianshulu (R. Mkandawire, personal communication 1996). Parasitological and serological prevalence figures from the Mamili survey area indicate that trypanosomal infections are probably acquired when cattle graze and water along the Kwando River. The possibility that tsetse challenge may occur when cattle or tsetse cross the Mamili target barrier cannot, however, be excluded. Considering these results, regular follow-up surveys are needed to monitor the possible spread of tsetse and trypanosomosis into the Mamili area of the Eastern Caprivi.

The parasitological and serological prevalence rates confirm the reports of recent trypanosomosis outbreaks in the Katima Mulilo area. According to the serological data, cattle from two sampling sites (Fooma and Mpacha) face regular tsetse challenge. During an extensive tsetse survey conducted in the

vicinity of Katima Mulilo no tsetse were trapped (P. van den Bossche, unpublished data 1995) although tsetse flies are present in the adjacent Sesheke area of Zambia. It is, therefore, assumed that trypanosomal infections are being acquired when cattle graze along the Zambezi River. Ufortunately, the low sensitivity of trapping methods for *G. m. centralis* makes it impossible to draw conclusions from tsetse survey results alone. Information obtained from the serological survey, however, clearly indicates that tsetse have not been able to establish themselves in Katima Mulilo and areas south of Katima Mulilo. Nevertheless, there will be a need for close vigilance in these areas until the threat of invasion from the north has been removed.

None of the animals sampled south of Katima Mulilo and in the Linyanti/Chobe survey area were infected with trypanosomes. These data are, however, not sufficient to conclude that the disease is absent. The seroprevalence of anti-trypanosomal antibodies was also low. Since the predictive value of a serological test declines as the prevalence of the disease declines, the low seroprevalence of anti-trypanosomal antibodies in cattle sampled south of Katima Mulilo and in the Linyanti/Chobe survey area could even be an overestimation of the true prevalence (Thrusfield 1986). Using the serological prevalence data and with the sample sizes used in this survey it can be assumed, with a high degree of confidence, that trypanosomosis is absent in those areas.

Of particular epidemiological interest is the difference in the prevalence of *T. vivax* infections in cattle from two areas despite their relatively close proximity. There may be several explanations. First, this could be explained by the occurrence of mechanical transmission although the role of biting flies in transmitting *T. vivax* to cattle in tsetse-infested areas remains an unresolved issue (D'Amico, Gouteux, Le Gall & Cuissance 1996). Second, may be differences in host availability or host preference between the two areas. Antelopes, which are abundant in the Mamili area, are generally accepted to be reservoir hosts of *T. vivax* from which the infection is transmissible to domestic ruminants (Hoare 1970). An increased feeding frequency by tsetse on antelopes could, therefore, result in a high *T. vivax* prevalence in cattle. Finally, for a tsetse fly to become infective, it must live longer than the developmental period of the trypanosome. Since *T. vivax* has the shortest developmental cycle, a high proportion of tsetse infected with *T. vivax* is expected in areas where large numbers of young tsetse flies are present. Proportionately larger numbers of younger flies relative to older flies may be recorded either when mortality is high in a relatively stable tsetse population, or when the mortality is low in an expanding tsetse population. The Mamili survey area is situated at the edge of the fly-belt. Ecological conditions for tsetse, at the edge of a fly-belt, are normally less favourable resulting in a high mortality rate of tsetse. This high mortality rate could favour the development of *T. vivax* infections in tsetse and could explain the high *T. vivax* prevalence rate in cattle sampled in the Mamili survey area.

Packed cell volume and trypanosomosis prevalence

Although anaemia can be caused by factors other than trypanosomosis, it remains one of the most important indicators of tsetse-transmitted trypanosomosis in cattle (Stephen 1986). The PCV-profile and average PCV of a herd is affected by the number of trypanosome-infected animals or the parasitological prevalence of trypanosomosis. This is clearly seen in the shift of the PCV distribution to the lower PCV-values in survey areas where trypanosomosis was detected (Fig. 2). This observation suggests that PCV-profiles can be used as an additional indicator of trypanosomosis even when trypanosomes could not be detected by parasitological diagnostic tests.

Interpretation of serological data

Contrary to the parasitological methods, the serological test used in this survey has high sensitivity and specificity. Nevertheless, interpreting anti-trypanosomal antibody prevalence rates remains difficult. This is mainly because such antibodies can persist for several months even after successful trypanocidal drug therapy or self-cure (Bocquentin, Very & Duvallet 1990). The effect of tsetse control measures on the transmission of bovine trypanosomosis is often assessed by determining the parasitological incidence of trypanosomosis in sentinel cattle. This type of surveillance is expensive and lacks sensitivity (Paris, Murray & Mcodimba 1982). Although antibody detection tests cannot form the basis of identifying infected animals (Nantulya 1990), a decline in antibody prevalence can also be used to assess the impact of tsetse control operation on the trypanosomosis challenge. Effectiveness of tsetse control measures can, therefore, be monitored by regular surveys to establish the prevalence of anti-trypanosomal antibodies. These types of surveys are easy to conduct, less time consuming than the normal surveillance and have high sensitivity and specificity. Once anti-trypanosomal antibodies have disappeared seroprevalence surveys can continue to be used as a sensitive monitoring system. Such a monitoring system is extremely useful in countries where tsetse-cleared areas are protected by, for example, target barriers to prevent re-invasion of tsetse from infested areas (Van den Bossche & Mudenge 1997).

Of primary interest when conducting this serological survey was to determine whether the population or herd has been exposed to trypanosomosis. In this

respect, areas of particular interest are those where disease prevalence is too low to detect parasites by current parasitological diagnostic methods or where tsetse cannot be captured. In such cases, the determination of the anti-trypanosomal antibody prevalence of a herd makes it possible to distinguish between low challenge and no challenge. One such example is the Katima Mulilo survey area where the trypanosomosis situation could only be explained by combining parasitological and serological data.

Although some animals with recent trypanosomal infections may not yet have developed antibody response at the time of sampling, 90 % of the parasitologically positive herds were also serologically positive. Moreover, herd true seroprevalence was positively correlated to parasitological prevalence and the percentage of anaemic animals. Those findings indicate that, on a herd basis, the prevalence of anti-trypanosomal antibodies can reflect the infection status or extent of disease.

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