CHAPTER THREE

BOVINE TRYPANOSOMOSIS IN MALAWI, NAMIBIA AND ZIMBABWE

3.1   Introduction

An important requirement for developing a strategy for the localised control of tsetse-transmitted bovine trypanosomosis is to have a clear picture of the extent and magnitude of the nagana problem and a basic understanding of its local epidemiology. For many decades, emphasis was placed on determining the distribution and density of the vector rather than the prevalence of the disease in cattle. This is not surprising in view of the past tsetse eradication policy and the absence of cattle in extensive areas of potential tsetse habitat. However, information on the distribution and density of tsetse does not suffice when developing a strategy for the localised control of tsetse-transmitted bovine trypanosomosis. There are several reasons for supplementing tsetse data with bovine trypanosomosis prevalence figures. First and foremost, using bovine trypanosomosis prevalence data areas where nagana is present or areas at risk can be identified. Moreover, areas can be classified according to the proportion of animals infected or levels of challenge and different epidemiological situations can be identified. This information can be used to identify priority areas for particular types of control. Second, disease prevalence data are essential in evaluating the effectiveness of control measures.

Surprisingly, accurate data of the distribution and prevalence of nagana in southern Africa are not available. This is partly due to the present entomological bias in the management of tsetse-transmitted trypanosomosis. Moreover, determining the prevalence of bovine trypanosomosis accurately is fraught with difficulties. The parasitological diagnostic methods in common use have low diagnostic sensitivity. Hence, a substantial proportion of the parasitologically positive animals will not be detected. This will result in an underestimate of the prevalence of infection. Consequently, an area cannot be declared disease-free on the basis of parasitological diagnostic tests alone. Moreover, trypanocidal drugs are used widely in most countries of the region. Parasitological diagnostic tests cannot distinguish between areas where the disease is absent and areas where the prevalence is low or where trypanocidal drugs are used effectively. Finally, in many countries of the southern African region, tsetse are confined to a particular habitat. The interaction between cattle and tsetse often occurs seasonally. This will not be detected easily by a one off

survey using parasitological diagnostic methods. The obvious shortcomings of the parasitological diagnostic method in determining the distribution of bovine trypanosomosis can be compensated for partly by surveillance. However, surveillance is time consuming, expensive and is, therefore, limited to areas of particular interest. Clearly, the methods currently used to determine the distribution and prevalence of bovine trypanosomosis need to be supplemented with more sensitive diagnostic tests.

The recently improved anti-trypanosomal antibody detection ELISA may be such a tool. The test was developed about 20 years ago (Luckins, 1977) but has hardly been used for extensive surveys. Recently, the assay was further developed for detection of anti-trypanosomal antibodies in eluted blood spots collected on filter papers. Anti-trypanosomal antibodies are an indirect indication of a trypanosomal infection and persist even after a trypanosomal infection has been cured. Whereas the persistence of antibodies is often considered a disadvantage, it may be advantageous under certain circumstances. Indeed, determining the prevalence of animals with anti-trypanosomal antibodies may identify areas where challenge is low or irregular. When assessing the effectiveness of control operations, on the other hand, an understanding of the dynamics of anti-trypanosomal antibodies after an infection has been cured is essential. A trial was conducted in eastern Zimbabwe to determine this decline in antibody levels after treatment (Section 3.2). Another factor, which may interfere with the interpretation of anti-trypanosomal antibody prevalence data, is non-specific cross-reactions with antibodies against other diseases. Such cross-reactions will lead to an overestimate of the antibody prevalence. Of particular importance, in this respect, are cross-reactions with antibodies against non-pathogenic trypanosomes (*T. theileri*) and/or antibodies against tick-borne parasites. The ELISA's species sensitivity with regard to *T. theileri* has already been assessed (Hopkins *et al.*, 1998). Tick-borne diseases, especially babesiosis and anaplasmosis, occur over large areas where tsetse are present. Non-specific cross-reactions with antibodies against these diseases, in their acute and/or latent phases, would seriously affect the ELISA's usefulness. Hence, a study was undertaken to determine if non-specific cross-reactions with antibodies against *Anaplasma marginale* and bovine *Babesia* spp. do occur (Section 3.3).

Promising preliminary results were obtained in eastern Zambia where a large-scale bovine trypanosomosis survey was conducted as part of the antibody ELISA's development and validation (Hopkins, 1997). Nevertheless, there is a need to use the test more widely in the Region. Therefore, both parasitological and anti-trypanosomal antibody detection diagnostic methods were used to determine the distribution of bovine trypanosomosis in Malawi (Section 3.4), Namibia (Section 3.5) and Zimbabwe (Section 3.6). Information on the parasitological prevalence of trypanosomosis and the prevalence of anti-trypanosomal antibodies in cattle was used to clarify the epidemiology of bovine trypanosomosis in each of these countries. Moreover, the usefulness of anti-trypanosomal antibody prevalence data in evaluating the effectiveness of tsetse control operations is assessed (Section 3.6).

3.2 The decline of anti-trypanosomal antibody levels in cattle after treatment with trypanocidal drugs and in the absence of tsetse challenge

3.2.1 Introduction

When determining the distribution, or studying the epidemiology, of bovine trypanosomosis use is usually made of parasitological diagnostic tests. These tests are simple but lack the diagnostic sensitivity required for an accurate assessment of the distribution of infected animals (Paris *et al.*, 1982). Recently, an anti-trypanosomal antibody-detection enzyme-linked immunosorbent assay (ELISA) was adapted for use with dried blood spots on filter paper (Hopkins *et al.*, 1998). The test has high diagnostic sensitivity and specificity. It has been used in large-scale bovine trypanosomosis surveys and for monitoring the effectiveness of tsetse control interventions in southern Africa. Although knowledge of the prevalence of anti-trypanosomal antibodies in cattle is very useful, interpretation of the results is often difficult. This is due to the persistence of anti-trypanosomal antibodies even after an animal has been cured (Bocquentin *et al.*, 1990). To facilitate the interpretation of data on anti-trypanosomal antibody prevalence in cattle, a study was undertaken to determine the changes in the antibody levels after treatment with trypanocidal drugs.

3.2.2 Materials and methods

3.2.2.1 Experimental animals

Anti-trypanosomal antibody levels were studied in a herd of adult Mashona breed cattle. They were kept under natural tsetse challenge (*G. m. morsitans* and *G. pallidipes*) in an area along the Zimbabwe/Mozambique border in Mudzi District (Mashonaland East Province), Zimbabwe. The monthly incidence of trypanosomosis in this area varied between months and was on average about 20%. Each month, blood taken from an ear vein of each animal was examined for trypanosomes using the haematocrit centrifuge, phase contrast technique (Murray *et al.*, 1977). Ear vein blood, contained in one heparinized microhaematocrit centrifuge capillary tube, was extruded onto a filter paper (Whatman n° 4, Whatman®). Eluted blood spots were screened for the presence of trypanosomal antibodies using an indirect anti-trypanosomal antibody detection ELISA (Hopkins *et al.*, 1998). Use was made of a *T.*

congolense (IL 3000) invariable antigen batch prepared by the Parasitology Laboratory of the Department of Paraclinical Studies of the School of Veterinary Medicine, University of Zambia. Each blood spot was analysed three times, on different plates. A rigorous system of quality assurance was adopted. The Optical Density (OD) of each ELISA sample tested was expressed as a percentage (percentage positivity) of the strong positive reference standard (Wright *et al.*, 1993). A cut-off of 28% positivity was used. Animals with a percentage positivity equal to or larger than 50% were treated with diminazene aceturate (Berenil®. Hoechst) at 7.0 mg/kg body weight and transferred immediately to a tsetse-free zone. Once an animal was transferred, blood spots continued to be collected at monthly intervals and were screened for anti-trypanosmal antibodies using the ELISA.

Two animals, one in the tsetse-infested and one in the tsetse-free area, were not transferred and served as controls. Blood spots were collected at monthly intervals and anti-trypanosomal antibody levels were determined as described above.

3.2.2.2 Data analysis

The decline in anti-trypanosmal antibody levels over time in each experimental animal was examined by regression analysis. The significance of the difference between the slope of the regression lines was tested by an analysis of variance (Sokal and Rohlf, 1998). The probability of an animal having anti-trypanosomal antibodies in consecutive months after treatment was calculated using a survival analysis (Bland, 1987). All analyses were performed using the statistical package SPSS (SPSS Inc.).

3.2.3 Results

The standard deviation of the repeated measurement of the percentage positivity of each sample was very small. Therefore, monthly averages were calculated of the repeated measures of each sample. The averages were used in the analysis.

3.2.3.1 Decline in percentage positivity in individual animals

(i) Control animals

During the 12 months observation period, the average percentage positivity of bloodspots collected from the control animal, in the tsetse-free zone, varied between months but never exceeded the cut-off value (Fig. 3.2.1). The control animal, kept in the tsetse-infested zone, developed anti-trypanosomal antibodies. It remained positive from month 5 onwards (Fig. 3.2.1).
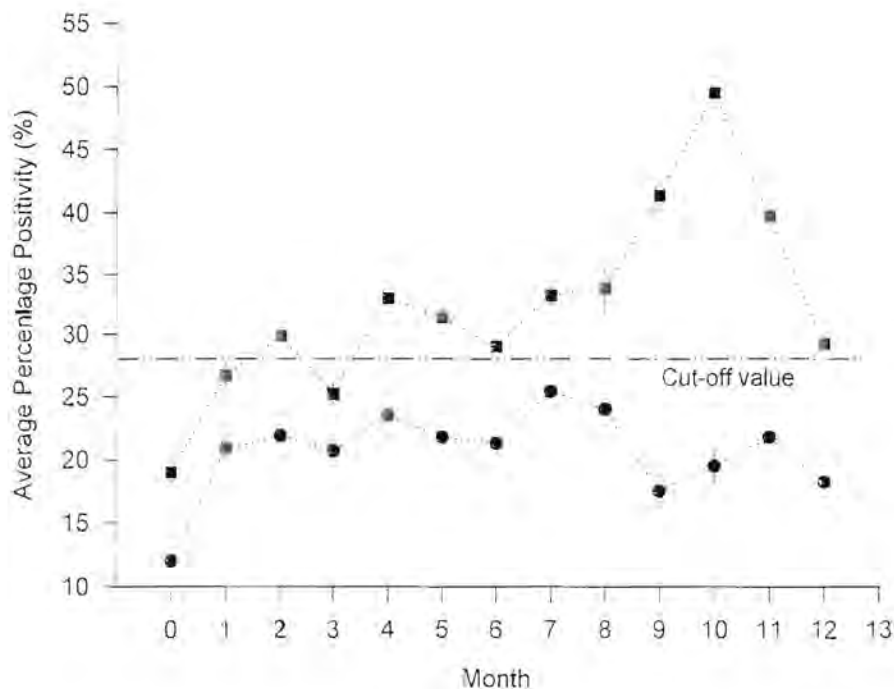

Figure 3.2.1: Average percentage positivity (%) (± 1 s.e) of the positive (■) and negative (●) control animal in consecutive months.

(ii) Animals transferred to tsetse-free zone

A total of 7 animals (henceforth referred to as animals 1 to 7) were treated and transferred from the tsetse-infested to the tsetse-free zone. The average percentage positivity of bloodspots at the moment of transfer was 70.6 ± 1.9%. The average percentage positivity declined rapidly in the absence of challenge and reached 37.1 ± 2.3% five months after transfer (Fig. 3.2.2). From month six onwards, the average

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percentage positivity continued to decline but at a lower rate. It reached a level lower than the cut-off value (28%), 13 months after treatment (Fig. 3.2.2). The decline of anti-trypanosomal antibodies was linear with a change in slope six months after treatment (Fig. 3.2.2). Therefore, the decline in average percentage positivity was considered over two periods, i.e. months 0 to 5 and months 6 to 14.
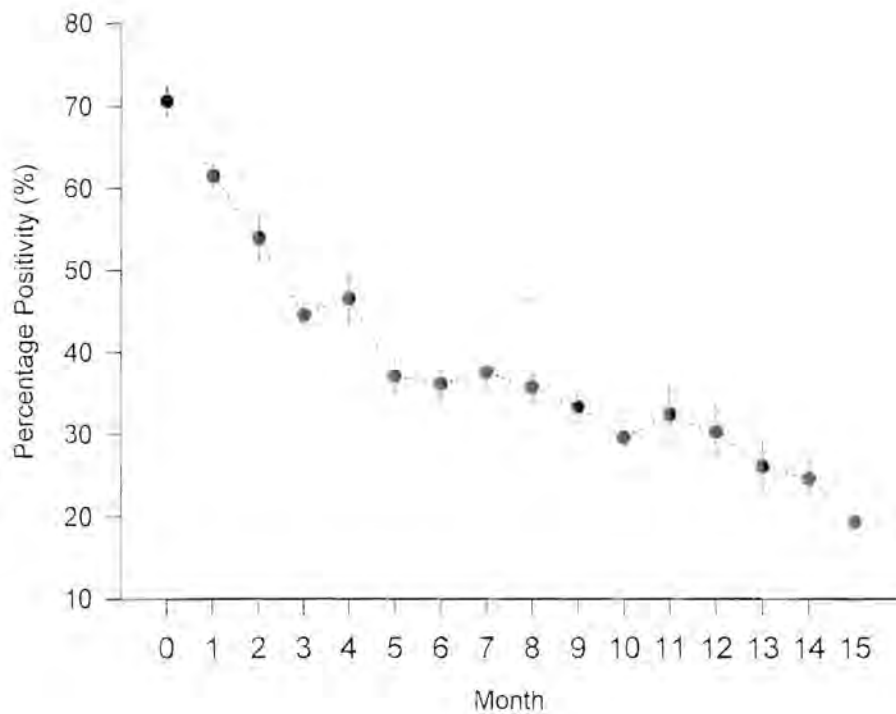

Figure 3.2.2: Average percentage positivity (%) (± 1 s.e.) in consecutive months of all experimental animals transferred to the tsetse-free zone.

3.2.3.2 Decline in percentage positivity during the first five months after transfer (Fig. 3.2.3)

The decline in the anti-trypanosomal antibody level, the first five months after treatment, was almost linear (Table 3.2.1). With the exception of animal 3, which had a temporary increase in anti-trypanosomal antibody level four months after treatment

(Fig. 3.2.3), the "time after treatment" explained between 91.4 and 98% of the variation in the anti-trypanosomal antibody level.

Table 3.2.1: Linear regression of anti-trypanosomal antibody level on months after treatment (months 0-5) for each of the experimental animals.

Animal	a	b	r	Significance
1	78.9	-9.1	0.98	P<0.001
2	76.8	-7.4	0.97	P<0.001
3	71.9	-5.2	0.65	P<0.004
4	70.8	-6.7	0.99	P<0.001
5	62.7	-8.1	0.96	P<0.001
6	60.4	-7.5	0.99	P<0.001
7	51.0	-4.7	0.96	P<0.001

a = intercept
b = regression coefficient
r = correlation coefficient

The decline in anti-trypanosomal antibody levels after treatment (quantified by the slope of the regression lines (b)) differed significantly between animals (Table 3.2.2) but was not affected by the antibody level at the moment of treatment ($r = -0.51$, P>0.05). The monthly decline in percentage positivity was, on average, 10% of the percentage positivity at the moment of treatment.
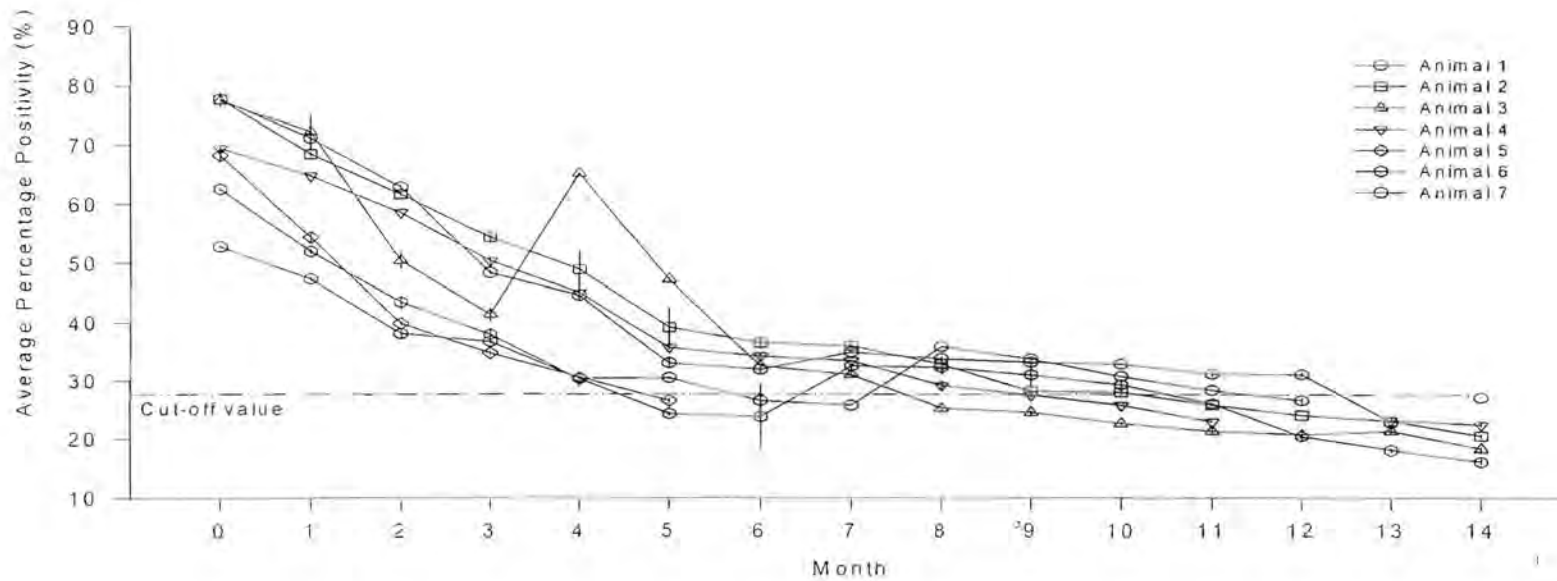
Figure 3.2.3: Average anti-trypanosomal antibody level (expressed as the average percentage positivity (%)) (± 1 s.e.) of animals 1-7 in consecutive months after treatment (month 0 being the month of treatment).

Table 3.2.2: Comparison between animals of the rate of antibody decline (slope of the regression lines) during the first five months after treatment.

	Animal						
	1	2	3	4	5	6	7
1	-	*	*	*	ns	*	*
2	*	-	ns	ns	ns	ns	*
3	*	ns	-	ns	ns	ns	ns
4	*	ns	ns	-	*	*	*
5	ns	ns	ns	*	-	ns	*
6	*	ns	ns	*	ns	-	*
7	*	*	ns	*	*	*	-

* = significant at the 0.05 level of P
ns = not significant

3.2.3.3 Decline in percentage positivity between months 6 and 14 after transfer (Fig. 3.2.3)

With the exception of animal 7, the anti-trypanosomal antibody level continued to decline between months 6 and 14 after treatment (Table 3.2.3). However, the rate of decline was substantially lower compared to the one observed during first five months.

Table 3.2.3: Table 2: Linear regression of anti-trypanosomal antibody level on months after treatment (months 6-14) for each of the experimental animals.

Animal	a	b	r	Significance
1	40.9	-1.0	0.67	P<0.01
2	48.9	-2.1	0.97	P<0.001
3	41.1	-1.7	0.93	P<0.001
4	43.1	-1.6	0.93	P<0.001
6	41.8	-1.6	0.68	P<0.001
7	34.8	-0.6	0.39	P>0.05

a = intercept
b = regression coefficient
r = correlation coefficient

The slope of the regression lines differed significantly between animals (Table 3.2.4) but was not affected by the antibody level at the moment of treatment ($r = -0.76$, P>0.05). For the majority of the animals the "time after treatment" explained most of the variation in the anti-trypanosomal antibody level (Table 3.2.3).

Table 3.2.4: Comparison between animals of the rate of antibody decline (slope of the regression lines) during months 6 and 14 after treatment.

	Animal					
	1	2	3	4	6	8
1	-	*	*	*	ns	ns
2	*	-	ns	*	ns	ns
3	*	ns	-	ns	ns	ns
4	*	*	ns	-	ns	ns
6	ns	ns	ns	ns	-	ns
8	ns	ns	ns	ns	ns	ns

* = significant at the 0.05 level of P
ns = not significant

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Between month 6 and month 14, the monthly average decline in percentage positivity was 3.6% of the average percentage positivity in month 6 after treatment.

3.2.3.4 Survival analysis

Approximately 50% of the seropositive animals became seronegative 7.5 months after treatment (Fig. 3.2.4). All animals had have become seronegative 13 months after treatment (Fig. 3.2.4).

3.2.4 Discussion

The level of anti-trypanosomal antibodies, in specimens collected from all experimental cattle, declined rapidly after treatment with trypanocides. A similar rapid decline in the level of anti-trypanosomal antibodies after treatment has been observed by other workers (Wilson and Cunningham, 1971; Luckins, 1977; Dwinger et al., 1988b: Bocquentin et al., 1990). Luckins (1977), using a microplate ELISA with various antigens, found that antibodies persisted up to 83 days after treatment with diminazene aceturate. Bocquentin et al. (1990), using an ELISA with T. congolense antigen, detected anti-trypanosomal antibodies up to 116 days after treatment but persistence differed significantly between experimental animals. All these results concur with those obtained using the indirect fluorescent antibody test (IFAT) to detect anti-trypanosomal antibody (Wilson and Cunningham, 1971; Zwart et al., 1973; Bocquentin et al., 1990). However, contrary to the above-mentioned work, the majority of the experimental animals were still seropositive after this initial phase of rapid decline in antibody level. It took another 8 months before the anti-trypanosomal antibodies had disappeared in all experimental animals. During this period (months 6 to 13), the antibody level continued to decline significantly though at a much lower rate.
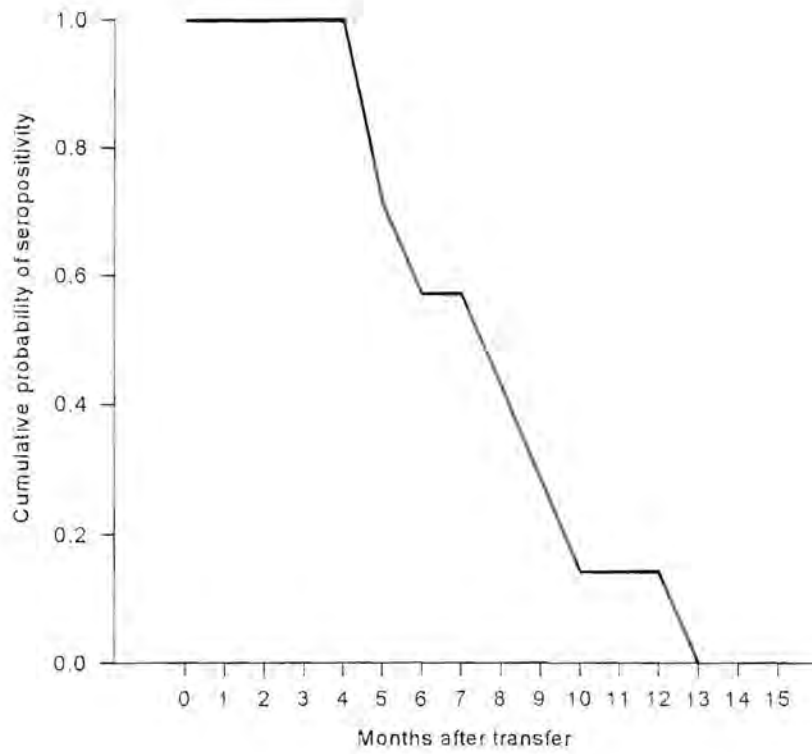
Figure 3.2.4: Cumulative probability of animals having anti-trypanosomal antibodies in consecutive months after treatment.

These data suggest that the persistence of anti-trypanosomal antibodies after treatment with trypanocidal drugs is much longer than has been suggested previously. This is in accordance with observations made by Authié *et al.* (1993) and Hopkins (1997). In both cases, anti-trypanosomal antibodies were present up to 10 months after treatment of a *T. congolense* infection with diminazene aceturate. The decline in the levels of anti-trypanosomal antibodies observed by Authié *et al.* (1993) was similar to the one in the present experiments. The antibody levels decreased progressively until Day 50 and remained higher than the pre-challenge levels for several months (Authié *et al.*, 1993). The question remains as to why the persistence of anti-trypanosomal antibodies is so much higher in the present study compared to those of many others. The most likely explanation is the difference in the sensitivity of the various tests. The high sensitivity of the test used in this experiment, compared to those used by other workers, can be explained as follows. First, the ELISA used in the present experiment made use of a *T. congolense* antigen for coating (Hopkins *et al.*, 1998). *Trypanosoma congolense* is the dominant trypanosome species in the area. Second, the cut-off value used in the present test was determined by making use of highly representative reference samples (Greiner *et al.*, 1997a). The cut-off value used for the antibody-ELISA used in this experiment was determined using blood spots collected from Mashona breed cattle kept in a tsetse-free area of Zimbabwe. The positive blood spots were collected from parasitologically positive animals in Zimbabwe and eastern Zambia. Hence, samples used to determine the cut-off value were obtained from representative reference populations. The accuracy of the cut-off can still be questioned. However, notwithstanding the fluctuations in the average percentage positivity of the negative control animal, the average percentage positivity never exceeded the cut-off value (28%). Moreover, during a five-month serosurveillance exercise conducted in the tsetse-free area, described above only five (2.2%) of a total of 222 animals had trypanosomal antibody values higher than the cut-off value. Both observations suggest that an appropriate cut-off value was used. Furthermore, if the experimental animals had been seronegative five months after treatment, the average percentage positivity would not be expected to decline over time but fluctuate around the same value as was the case in the negative control animal.

The observed dynamics of the anti-trypanosomal antibody levels in cattle after treatment with trypanocides have important practical implications. Sentinel herds of cattle are often used when monitoring the effectiveness of vector control operations. These herds are followed up at regular intervals and the parasitological incidence of trypanosomosis is determined. The value of this type of monitoring depends largely on the sensitivity of the diagnostic tests. Because of the low diagnostic sensitivity of tests for the parasitological diagnosis of trypanosomosis, results from such surveillance exercises should be interpreted with caution. The apparent absence of an infection does not necessarily mean the complete absence of challenge. More sensitive diagnostic methods are required to establish unequivocally the effect of the control intervention. The anti-trypanosomal antibody detection ELISA may offer this possibility. The present results have shown that, in the absence of challenge, the levels of anti-trypanosomal antibodies decline steeply in animals treated with trypanocidal drugs. Hence, the establishment of sentinel herds consisting of seropositive cattle that have been treated with trypanocides and the follow-up of the decline in the anti-trypanosomal antibody level over time may be a useful adjunct to evaluating the effectiveness of a tsetse control intervention.

3.3 An investigation of non-specific cross reactions in an anti-trypanosomal antibody detection ELISA for the diagnosis of bovine trypanosomosis

3.3.1 Introduction

The anti-trypanosomal antibody detection enzyme-linked immunosorbent assay (antibody-ELISA) has high diagnostic sensitivity and specificity (Luckins, 1977) which makes it a useful tool to supplement parasitological diagnostic methods that have variable, but generally low, sensitivity (Paris *et al.*, 1982; Desquesnes and Tresse, 1996). The diagnostic sensitivity may be affected by the occurrence of non-specific reactions. For example, the capillary tube agglutination test for measuring anti-trypanosomal antibodies in bovine and human serum gave positive reactions in cattle infected with *Theileria* spp. (Robson, 1972). When developing and evaluating the anti-trypanosomal antibody detection ELISA for bovines, the possibility of non-specific cross reactions was investigated using sera infected with various parasites (Luckins, 1977). Recently, the antibody ELISA was further developed for the analysis of blood samples collected on filter paper (Hopkins *et al.*, 1998). Although this new version of the antibody ELISA distinguishes between pathogenic and non-pathogenic (*T. theileri*) trypanosomes (Hopkins *et al.*, 1998), further research in non-specific cross reactions is required. Tick-borne diseases of cattle, especially anaplasmosis and babesiosis, are very common in many areas of southern Africa where tsetse-transmitted trypanosomosis is present. Non-specific cross reactions between the antibodies against those tick-borne parasites and the antibodies developed against trypanosomes would obviously result in misinterpretation of trypanosomosis survey and surveillance data. Therefore, the occurrence of such cross reactions in the acute and chronic phase of anaplasmosis (*Anaplasma marginale*) and babesiosis (*Babesia bigemina*) was investigated.

3.3.2 Materials and methods

3.3.2.1 Experimental animals

Blood samples were collected from Mashona breed adult cattle in Mudzi District (Mashonaland East Province) of Zimbabwe. Trypanosomosis-positive samples were obtained from cattle in areas immediately adjacent to the Mozambique

border where tsetse (*G. m. morsitans* and *G. pallidipes*) are present. Samples from the parasitologically negative and tick-borne parasite infected animals were obtained from cattle kept in areas cleared of tsetse immediately west of a target barrier that prevents tsetse from re-invading (Section 5.6). In these areas, trypanosomosis was still present but the incidence of trypanosomal infections was very low (Section 5.6). Tick control was irregular. The main tick-borne diseases in the sampling area are those caused by *Anaplasma marginale* or *Babesia bigemina* (Norval *et al.*, 1983). Heartwater and theileriosis were virtually absent (Norval *et al.*, 1985; Peter *et al.*, 1998).

In the first phase of the experiment, a survey was conducted in the two areas. Since no distinction could be made between acute or chronic tick-borne infections, a second phase was initiated in March 1999. During this phase (March -August 1999), two sentinel herds each of 62 adult Mashona breed cattle were established. One herd was based in the tsetse-infested area along the Mozambique border and the other was herded in the area protected by the target barrier. Sentinel animals were sampled at monthly intervals to detect new (acute) tick-borne infections. Parasitologically positive (trypanosomosis or tick-borne diseases) sentinel animals were treated when the packed cell volume (PCV) was lower than 20%.

3.3.2.2 Diagnostic methods

Parasitological and serological methods were used for diagnosis. Blood was collected from an ear vein into heparinized microhaematocrit centrifuge capillary tubes and onto glass slides, as thick and thin blood smears. The capillary tubes were sealed with "Cristaseal" (Hawksley) and centrifuged immediately in a microhaematocrit centrifuge for 5 min. at 9 000 rpm. After centrifugation, the PCV was determined. Animals with a PCV $\leq$ 24% were considered to be anaemic. The buffy coat and the uppermost layer of red blood cells of each specimen 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 x 100 oil immersion objective lens for the presence of *Trypanosoma* spp., *A. marginale* or *B. bigemina*.

From most of the animals, blood contained in one heparinized microhaematocrit centrifuge capillary tube was extruded onto a filter paper (Whatman n° 4, Whatman®). Eluted blood spots were screened for the presence of trypanosomal antibodies using an indirect ELISA (Hopkins *et al.*, 1998). A rigorous system of quality assurance was adopted. The Optical Density (OD) of each ELISA sample tested was expressed as a percentage (percentage positivity, PP) of the strong positive reference standard (Wright *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 99.0%.

3.3.2.3 Statistical analysis

Samples were divided into four groups, i.e. parasitologically negative, *Trypanosma*-infected, *Anaplasma*-infected and *Babesia*-infected. A distinction was made between samples collected during the survey and those collected during surveillance. The average PCV and the percentage positivity of the four groups were compared using parametric or non-parametric statistical tests (Sokal and Rohlf, 1998). All statistical analyses were performed using the statistical package SPSS (SPSS Inc.).

3.3.3 Results

A total of 1 369 blood samples was collected. The average PCV differed significantly between the four groups ($P<0.01$ for samples collected during the survey and during surveillance) (Table 3.3.1 and Fig. 3.3.1). The PCV was highest in the parasitologically negative group and lowest in the animals infected with tick-borne parasites.

Table 3.3.1: Average PCV (± 1 s.e.) of parasitologically negative, trypanosome-infected and tick-borne parasite-infected samples collected during the survey and the surveillance.

Disease status	Survey		Surveillance	
	n	Average PCV (± 1 s.e.)	n	Average PCV (± 1 s.e.)
Negative	665	28.5 ± 0.3	513	30.4 ± 0.1
Trypanosoma spp.	36	26.4 ± 0.4^a	23	26.7 ± 1.2^a
A. marginale	60	23.4 ± 0.6^b	51	25.1 ± 0.6^a
B. bigemina	12	25.0 ± 1.1^{ab}	9	24.3 ± 1.3^a

Averages followed by the same letter are not significantly different at P<0.05.

A total of 1 251 blood spots was analysed for the presence of anti-trypanosomal antibodies. The average percentage positivity was significantly higher in the trypanosome-infected (92% *T. congolense*) group. It did not differ between parasitologically negative animals and animals infected with tick-borne parasites (Table 3.3.2 and Fig. 3.3.2). The differences in average percentage positivity of samples collected from cattle with tick-borne parasites, during the survey or during the surveillance, were not significant.

Table 3.3.2: Average percentage positivity (± 1 s.e.) of samples collected from parasitologically negative, trypanosome-infected and tick-borne parasite-infected cattle during the survey and the surveillance.

Disease status	Survey		Surveillance	
	n	Average PP (± 1 s.e.)	n	Average PP (± 1 s.e.)
Negative	665	19.2 ± 0.3^a	401	19.3 ± 0.3^a
Trypanosma spp.	36	39.8 ± 1.8	23	34.8 ± 1.9
A. marginale	60	18.8 ± 0.6^a	46	19.8 ± 0.9^a
B. bigemina	12	20.3 ± 2.1^a	8	19.2 ± 2.9^a

Averages followed by the same letter are not significantly different at P<0.05.

The majority (91.5%) of the samples collected from trypanosome-infected cattle had anti-trypanosomal antibodies (Table 3.3.3). The proportion of cattle, infected with *A.*

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marginale or *B. bigemina*, with anti-trypanosomal antibodies was low (9.5%) and differed little from the proportion of parasitologically negative animals with anti-trypanosomal antibodies (10.1%) (Table 3.3.3).

Table 3.3.3: Number of samples with anti-trypanosomal antibodies collected from parasitologically negative, trypanosome-infected and tick-borne parasite-infected cattle during the survey and the surveillance.

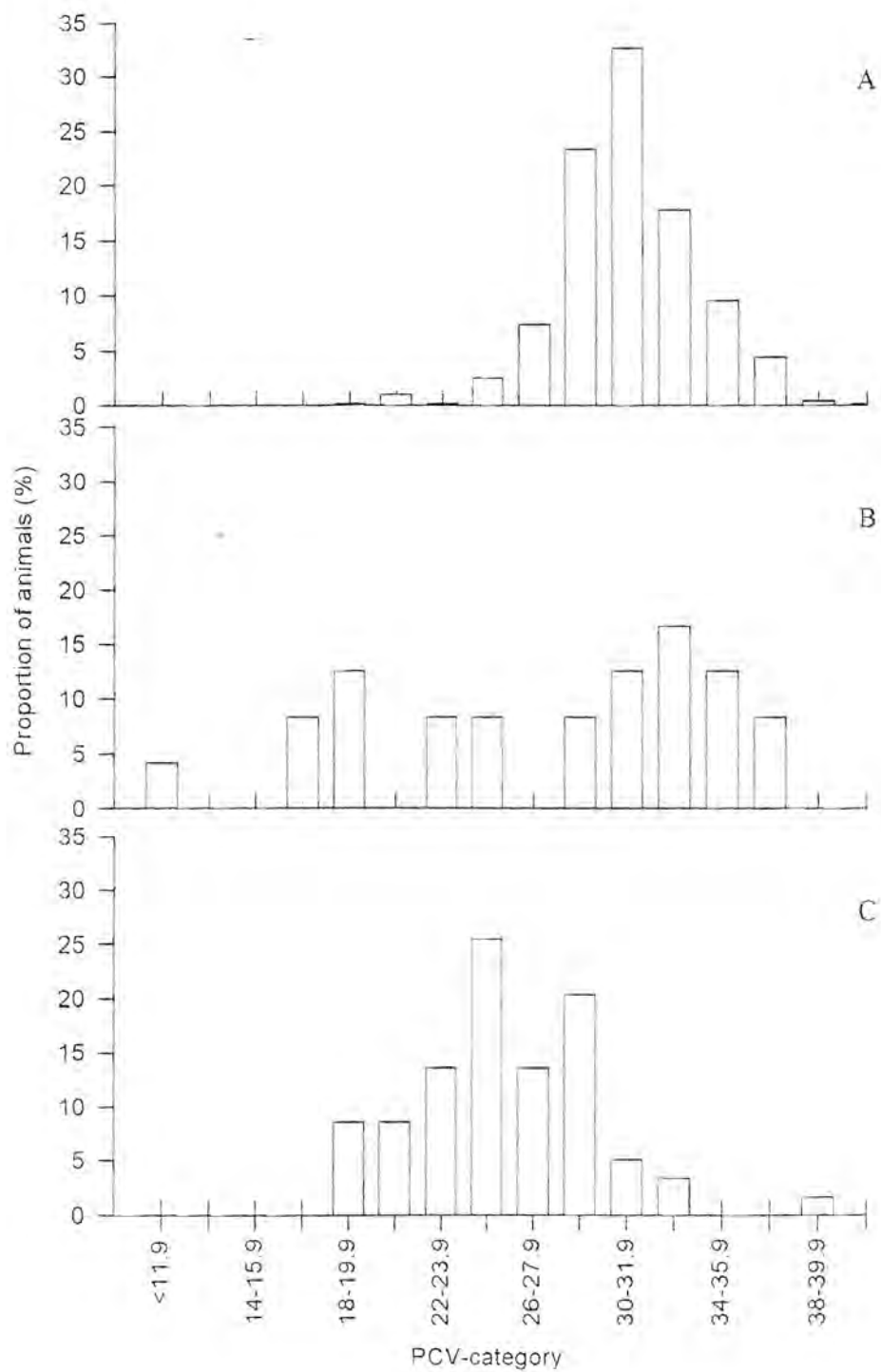| Disease status	Survey		Surveillance	
	Number analysed	Number positive (%)	Number analysed	Number positive (%)
Negative	665	59 (8.9)	401	49 (12.2)
Trypanosoma spp.	36	34 (94.4)	23	20 (86.9)
A. marginale	60	2 (3.3)	46	7 (15.2)
B. bigemina	12	2 (16.7)	8	1 (12.5)

Figure 3.3.1: PCV-distribution of parasitologically negative (A), trypanosome infected (B) and tick-borne parasite-infected (C) sentinel cattle.
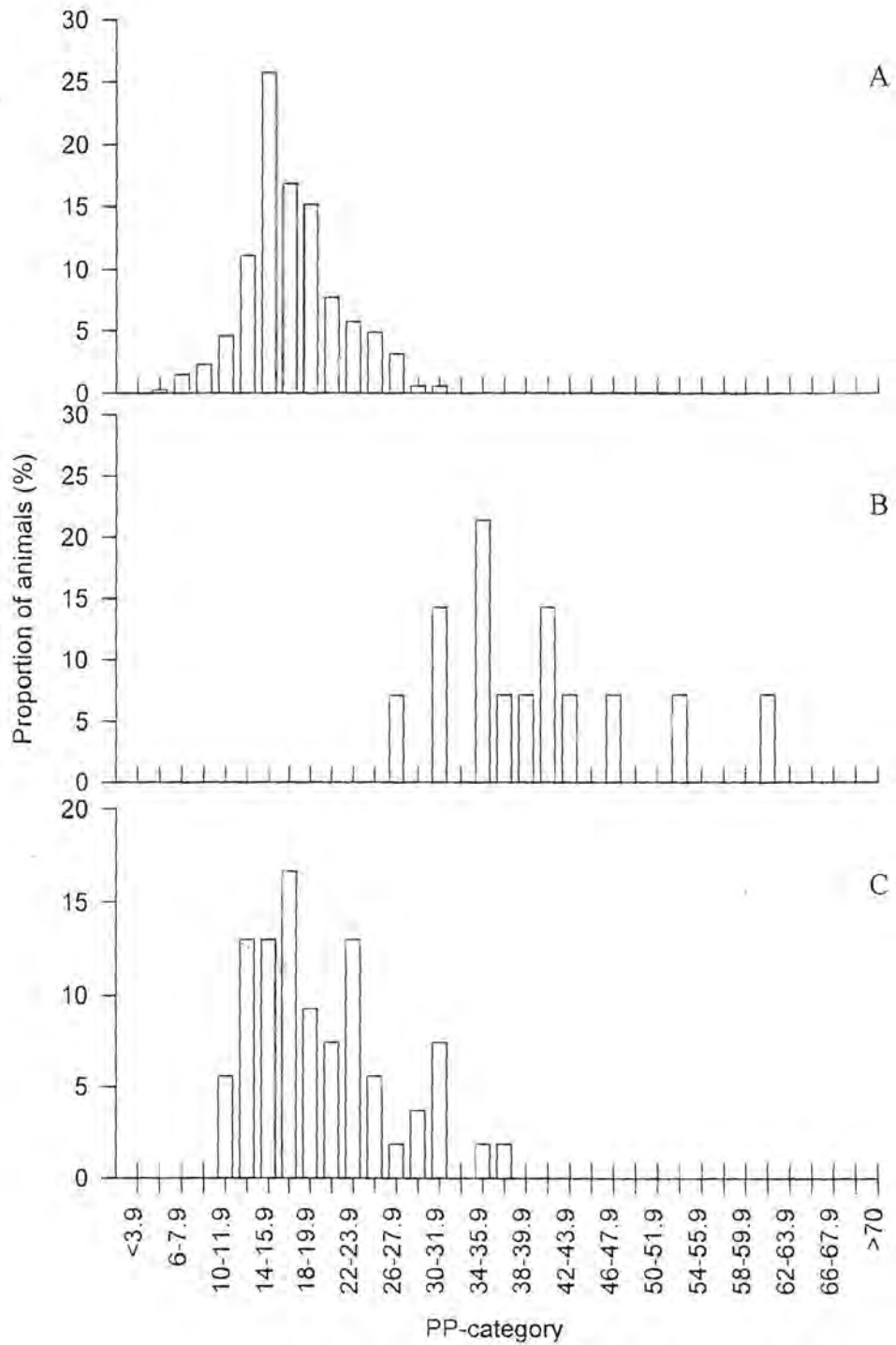
Figure 3.3.2: Distribution of the percentage positivity of samples collected from parasitologically negative (A), trypanosome-infected (B) and tick-borne parasite-infected (C) sentinel cattle.

3.3.4 Discussion

The results of this study show that the presence of a *B. bigemina* and *A. marginale* infection did not result in non-specific cross reactions. Although some animals infected with tick-borne parasites did react positively on the ELISA, the proportion of seropositives in the tick-borne parasite infected groups differed little from the proportion of seropositives in the parasitologically negative group. This observation suggests that the reasons for the occurrence of anti-trypanosomal antibodies in the cattle infected with tick-borne parasites do not differ from the reasons for the occurrence of anti-trypanosomal antibodies in the parasitologically negative animals. Such reasons could be false positive reactions and, since trypanosomosis is not completely absent, trypanosomal infections that were not detected or persistent anti-trypanosmal antibodies. It was difficult to establish if the cattle found to be infected with *Babesia* or *Anaplasma* during the survey were in the acute of chronic phase of infection. Progressive anaemia is a typical sign of patent *Babesia* and *Anaplasma* infections in cattle (de Vos and Potgieter, 1994; Potgieter and Stoltsz, 1994). The low average PCV of both the *Anaplasma*-and *Babesia*-infected group, therefore, suggests that a substantial proportion of the infected cattle must have been in the acute phase of the infection. Since sentinel cattle were sampled at monthly intervals, they were in the acute phase of the *Babesia* or *Anaplasma* infection upon the detection of the parasites. Despite the presence of this acute tick-borne infection, the proportion of sentinel cattle with anti-trypanosomal antibodies differed little from the proportion of parasitologically negative sentinel animals that had anti-trypanosomal antibodies. Hence, results show that non-specific cross reactions with antibodies against *A. marginale* and *B. bigemina* were absent in both the acute or latent stages of both tick-borne diseases.

This study showed that the ELISA was highly sensitive for trypanosomal infections. The majority of the animals infected with trypanosomes were identified by the anti-trypanosomal antibody ELISA. These results confirm the value of the test in trypanosomosis surveys.

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3.4 The distribution and epidemiology of bovine trypanosomosis in Malawi

3.4.1 Introduction

Tsetse have been reported in Malawi since the end of the 19th century (Austen, 1903). Nevertheless, the distribution of bovine trypanosomosis in Malawi was only mapped in the late 1980s (Davison, 1990). This national survey used parasitological diagnostic methods and revealed a disease distribution pattern that in most areas was correlated with the distribution of the vector. Unfortunately, the parasitological diagnostic methods for trypanosomosis have relatively low sensitivity (Paris *et al.*, 1982). Hence, many areas where the disease is present at low prevalence or where trypanocidal drugs are used frequently may not be detected and may thus be considered disease-free. Similarly, the sampling methods for tsetse are heavily biased and lack sensitivity especially for *G. m. morsitans* and *G. brevipalpis* two species present in Malawi (Hargrove, 1980b).

To improve the accuracy of bovine trypanosomosis distribution maps, an anti-trypanosomal antibody detection Enzyme-Linked Immunosorbent Assay (ELISA) (Luckins, 1977) was recently further developed for use in large-scale surveys (Hopkins *et al.*, 1998). The test has high sensitivity and specificity. It has the advantage that it detects antibodies against current and past trypanosomal infections. This makes it possible to identify areas where tsetse challenge is seasonal, where tsetse are present but cannot be detected and/or where trypanocidal drugs are used frequently.

To support the development of a strategy for the control of tsetse-transmitted trypanosomosis in Malawi a survey was conducted to update the distribution of bovine trypanosomosis. Use was made of parasitological and serological diagnostic methods. This section summarizes the findings of this survey. The usefulness of anti-trypanosomal antibody prevalence data is discussed and the findings of the survey are used to clarify the epidemiology of bovine trypanosomosis in Malawi.

3.4.2 Material and methods

3.4.2.1 Sampling sites and sample selection

Between August 1995 and June 1997 a total of 9 309 adult cattle were examined at 159 sampling sites (henceforth referred to as herds). Sampling was restricted to areas where cattle were present permanently and attempts were made to distribute the sampling sites evenly over 23 districts in the Northern, Central and Southern Regions of Malawi. A cross-sectional sampling method was applied. Sample sizes were calculated according to Cannon and Roe (1982) and depended on the total cattle population at a particular sampling site but never exceeded 60 head of cattle at a single sampling site. Sample sizes were calculated to provide 95% certainty of detecting at least one positive case at a prevalence of 5%.

3.4.2.2 Diagnostic methods

The buffy coat, stained thick and stained thin smear were used as parasitological diagnostic tests (Section 3.3.2.2).

From most of the animals from 150 herds, blood contained in one heparinized microhaematocrit centrifuge capillary tube was extruded onto a filter paper (Whatman n° 4, Whatman®). Eluted blood spots were screened for the presence of trypanosomal antibodies using an indirect ELISA (Section 3.3.2.2). All statistical analyses were performed using the statistical package SPSS (SPSS Inc.).

3.4.3 Results

3.4.3.1 Parasitological prevalence of bovine trypanosomosis

A total of 186 trypanosomal infections (1.9%) was diagnosed in cattle from 27 herds. The majority of infections were *T. congolense* (Table 3.4.1).

Table 3.4.1: Species prevalence of trypanosomal infections in cattle sampled in Malawi.

Trypanosome species	Number of infections	Trypanosome species prevalence (%)
T. congolense	176	94.6
T. vivax	9	4.8
T. brucei	1	0.6

The parasitological herd prevalence of trypanosomosis varied between 1.7% and 42.8% (on average $12.2 \pm 2.1\%$).

3.4.3.2 Prevalence of anti-trypanosomal antibodies

A total of 966 blood spots out of a total of 6 810 samples (14.2%) had anti-trypanosomal antibodies (Table 3.4.2). The majority of parasitologically positive animals were seropositive (80.8%) and 12.9% of the parasitologically negative animals had anti-trypanosomal antibodies (Table 3.4.2).

Table 3.4.2: Number of parasitologically negative and positive animals with and without anti-trypanosomal antibodies.

Parasitologically	Serologically		Total
	Positive (%)	Negative (%)	
Positive	105 (80.8)	25 (19.2)	130
Negative	861 (12.9)	5819 (87.1)	6680

Only 6 out of 9 parasitologically positive samples (66.7%) of the animals infected with *T. vivax* had anti-trypanosomal antibodies whereas 99 out of 120 parasitological positive samples (82.5%) of the animals with a *T. congolense* infection were seropositive. The average percentage positivity was much higher in serologically

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positive and parasitologically positive animals (53.7 ± 3.3%) compared to serologically positive but parasitologically negative animals (41.1 ± 0.7%).

The majority of the parasitologically positive herds (92.4%) were seropositive whereas 71.8% of the parasitologically negative herds were seropositive (Table 3.4.3). The prevalence of trypanosomal infections in the parasitologically positive herds was significantly correlated with the proportion of animals with anti-trypanosomal antibodies (r = 0.28, P<0.01) in those herds and their average percentage positivity (r = 0.29, P<0.01). Only 5.4% of all serologically negative herds contained animals with trypanosomal infections (Table 3.4.3).

Table 3.4.3: Number of parasitologically negative and positive herds with animals with anti-trypanosomal antibodies.

	Serologically		Total
Parasitologically	Positive (%)	Negative (%)	
Positive	24 (92.4)	2 (7.7)	26
Negative	89 (71.8)	35 (28.2)	124

3.4.3.3 Packed cell volume

The average PCV of parasitologically negative animals (31.4 ± 0.05%) was significantly higher (P<0.001) than the average PCV of parasitologically positive animals (22.5 ± 0.5%) (Fig. 3.4.1). It increased with decreasing percentage positivity (r = -0.12, P<0.001). Similarly, the average PCV was significantly lower in seropositive (30.1 ± 0.2%) compared to seronegative animals (31.9 ± 0.07%) (P<0.001). The average PCV of parasitologically negative animals that were also seronegative was significantly higher than the average PCV of parasitologically negative cattle in which anti-trypanosomal antibodies were detected (P<0.001) (Table 3.4.4).
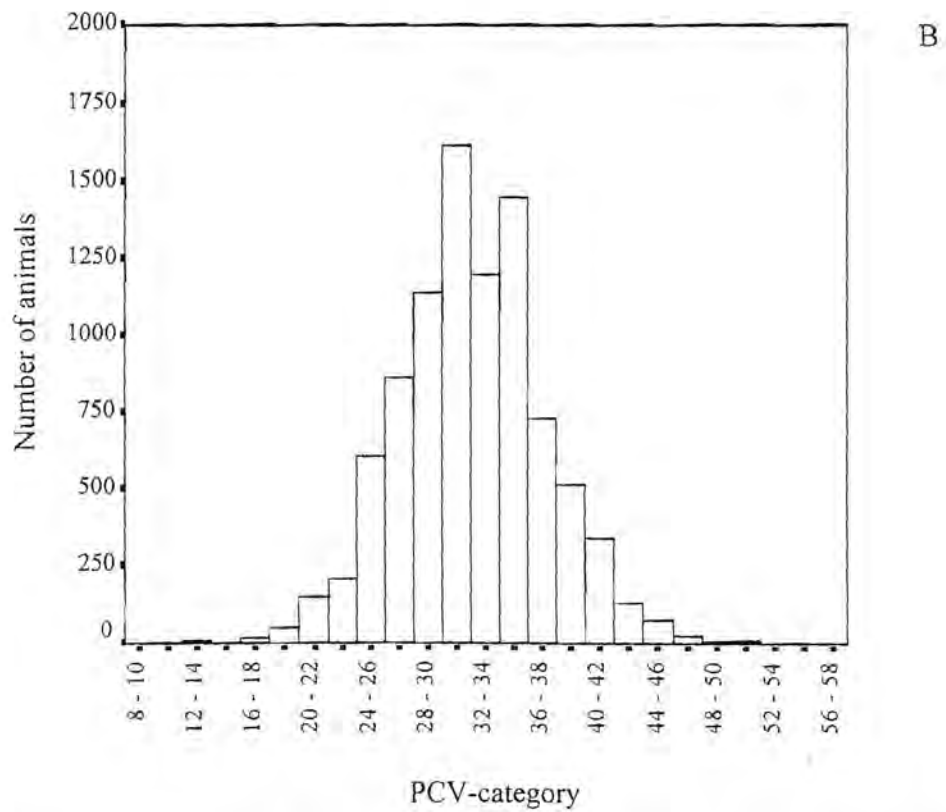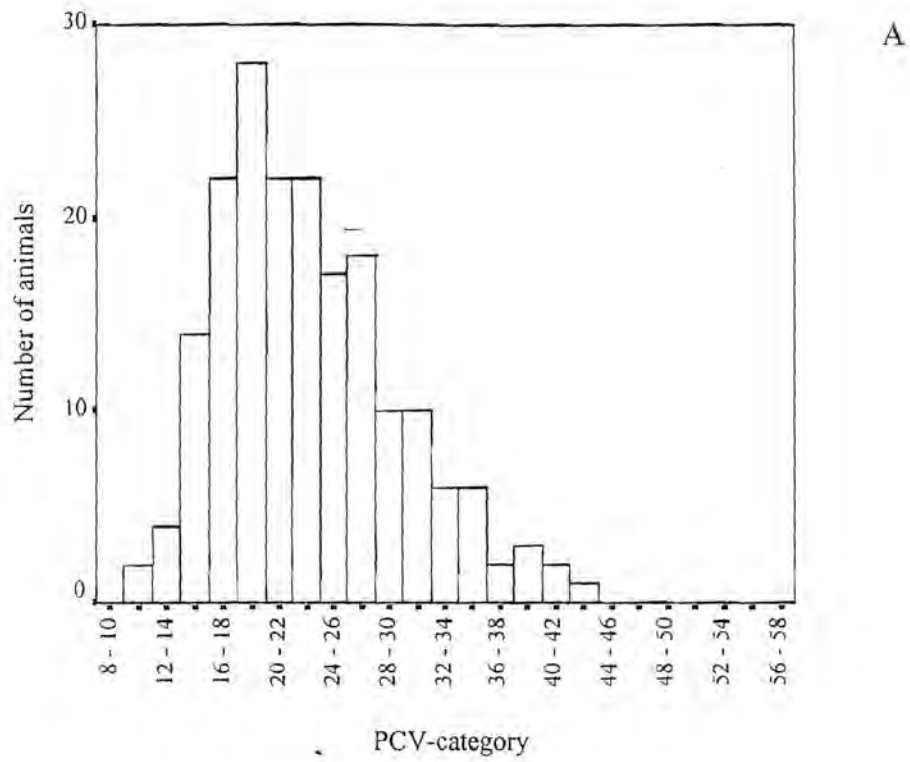
131

Figure 3.4.1: PCV-profile of parasitologically positive (A) and parasitologically negative (B) animals.

Table 3.4.4: Average PCV (% ± 1 s.e.) of serologically and parasitologically positive and negative animals.

Parasitologically	Serologically	
	Positive	Negative
Positive	21.7 ± 0.8^a	22.8 ± 0.6^a
Negative	30.6 ± 0.2	31.7 ± 0.1

Averages followed by the same letter are not significantly different at P<0.05 (Tukey-Kramer test)

The herd average PCV was significantly lower in parasitologically positive herds compared to parasitologically negative herds (29.9 ± 0.44% compared to 31.7 ± 0.18, P<0.001) and was significantly, negatively correlated with the prevalence of infection (r = -0.66, P<0.001,) (Fig. 3.4.2). It was highest in parasitologically and serologically negative herds (Table 3.4.5).

Table 3.4.5: Average PCV (% ± 1 s.e.) of serologically and parasitologically positive and negative herds.

Parasitologically	Serologically	
	Positive	Negative
Positive	29.8 ± 0.5^b	31.6 ± 0.3^{ab}
Negative	31.7 ± 0.2^{ab}	31.9 ± 0.3^a

Averages followed by the same letter are not significantly different at P<0.05 (Tukey-Kramer test)
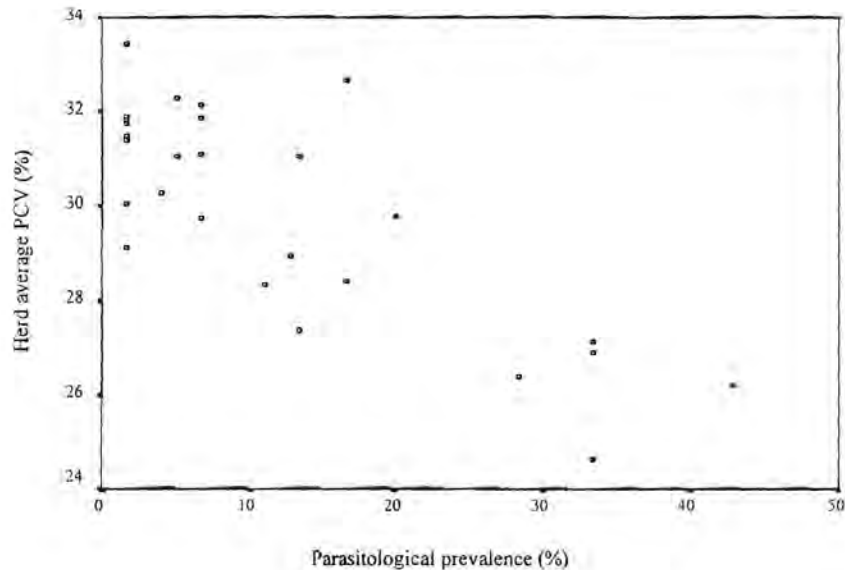
Figure 3.4.2: Scatterplot of the relationship between parasitological prevalence of trypanosomosis and herd average packed cell volume.

The herd average PCV of parasitologically negative but seropositive herds decreased with increasing average percentage positivity (r = -0.24, P<0.05) but was not correlated with the proportion of seropositive animals in the herd.

3.4.3.4 Distribution of bovine trypanosomosis in Malawi

(i) Northern Region

Several bovine trypanosomosis foci were identified in the Northern Region (Table 3.4.6 and Fig. 3.4.3).

Trypanosomal infections were detected in animals sampled in Rumphi (*T. congolense* (9), *T. vivax* (2), *T. brucei* (1) and mixed (1)) and Mzimba (*T. congolense* (9) and *T. vivax* (1)) Districts. In Rhumpi District, parasitologically positive herds were located within 10-15 km from the edge of the Vwaza Game Reserve (Fig. 3.4.3). Anti-trypanosomal antibodies, on the other hand, were found in animals sampled up to 40 km east of the Game Reserve. The proportion of animals with anti-trypanosomal antibodies was high (on average 32.1 ± 10.7%) in herds sampled along the Zambian

border south west of Mzimba along the South Rukuru river (Fig. 3.4.3). However, no trypanosomal infections were detected using parasitological diagnostic tests. In Karonga and Chitipa Districts, no trypanosomal infections were detected and the prevalence of anti-trypanosomal antibodies was generally low (on average $5.0 \pm 1.7\%$). One exception, however, was Mwangurukuru crushpen, situated close to Lake Malawi along the border with Tanzania (Fig. 3.4.3), where the prevalence of animals with anti-trypanosomal antibodies was high (75.1%). No trypanosomal infections were detected in cattle sampled along the shores of Lake Malawi. The prevalence of anti-trypanosomal antibodies was relatively high in cattle sampled at Chonanga and Chimyanga crushpens (28.2% and 26.5%, respectively), immediately east of the Nyika National Park (Fig 3.4.3).

Table 3.4.6: Average PCV (%), parasitological and anti-trypanosomal antibody prevalence (%) in each of the districts surveyed in the Northern Region of Malawi.

District	Number of herds	Average PCV (% ± 1 s.e.)	Parasitology		Serology	
			Number positive (%)	Number sampled	Number positive (%)	Number sampled
Chitipa	10	31.1 ± 0.2	0 (0)	600	11 (3.0)	364
Karonga	10	31.1 ± 0.2	0 (0)	600	45 (11.1)	404
Mzimba	13	30.7 ± 0.2	10 (1.1)	899	183 (26.3)	695
Nkhata Bay	2	32.1 ± 4.5	0 (0)	120	4 (4.6)	86
Rumphi	9	31.3 ± 0.2	13 (2.7)	481	134 (34.2)	392

Figure 3.4.3: Herd average PCV, proportion of anaemic animals, parasitological and serological prevalence of bovine trypanosomosis in the Northern Region of Malawi.
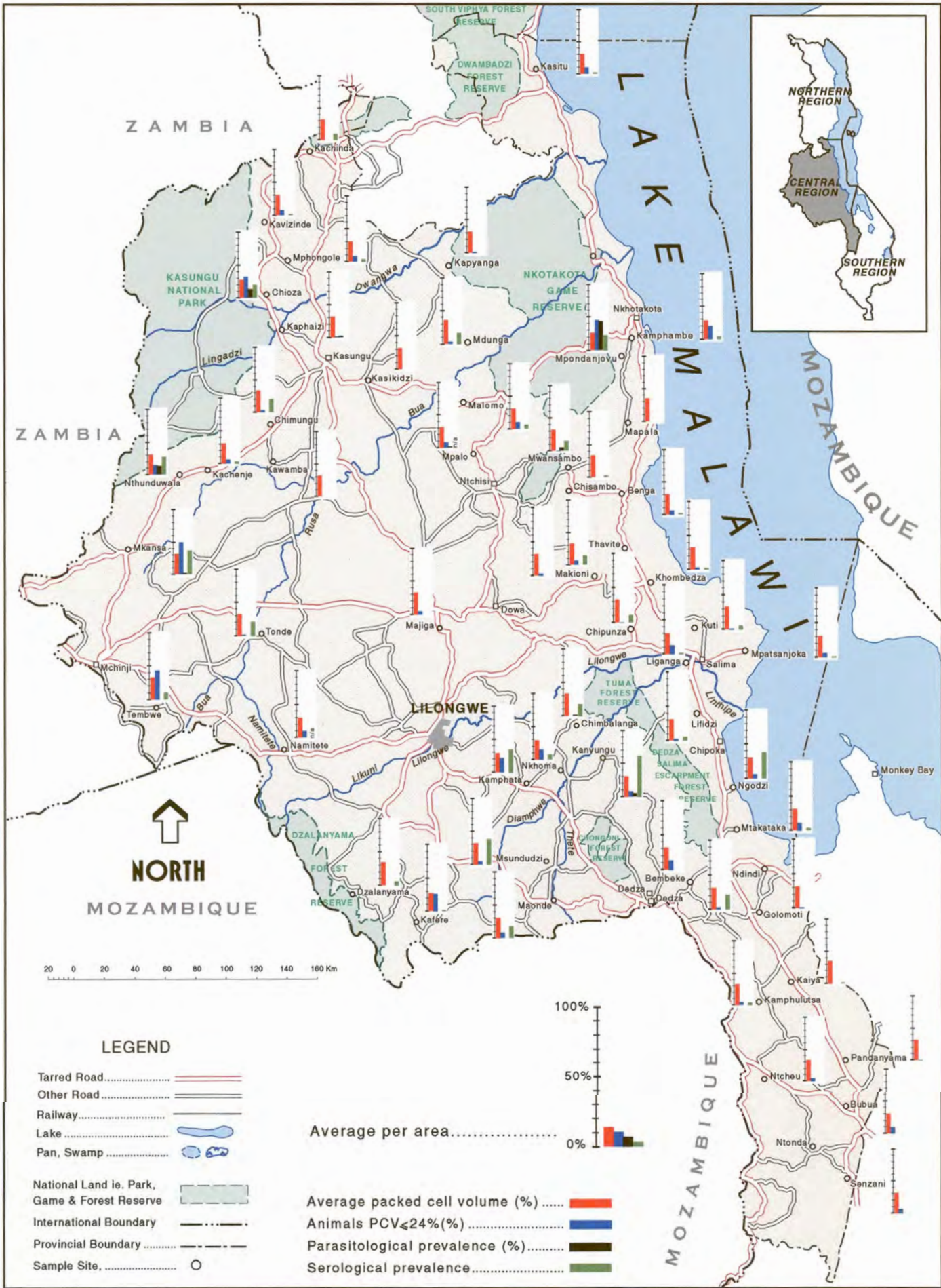
(ii) Central Region

In the Central Region, trypanosomal infections were detected in cattle sampled in the vicinity the Kasungu National Park and the Nkhotakota Game Reserve (Table 3.4.7 and Fig. 3.4.4). However, the prevalence of infection and the prevalence of cattle with anti-trypanosomal antibodies were generally low. The parasitological prevalence of trypanosomosis (42.8%, all *T. congolense*) and the proportion of anaemic animals (45.2%) was highest in cattle sampled at Mpondanjovu crushpen situated between the Nkhotakota Game Reserve and the shore of Lake Malawi (Fig. 3.4.4). Along the boundary of Kasungu National Park, trypanosomal infections were diagnosed in animals sampled at Chioza (8 *T. congolense* (13.7%)), Kaphaizi (1 *T. vivax* (1.7%)) and Nthunduwala crushpens (8 *T. congolense* (13.3%)) all situated within a 10-15 km wide band along the edge of the National Park (Fig. 3.4.4). The prevalence of anti-trypanosomal antibodies in cattle sampled in this band, was $11.3 \pm 4.2\%$. In Lilongwe and Dedza Districts, trypanosomal infections (*T. congolense* (3) and *T. vivax* (1)) were found in cattle sampled along the Tuma Forest Reserve and the Dedza-Salima Escarpment Forest Reserve (Fig. 3.4.4). The prevalence of animals with anti-trypanosomal antibodies was generally high along the Lilongwe-Dedza road (Kanyungu (62.0%), Msundudzi (47.6%) and Kamphata (34.6%)). With the exception of animals sampled at Ngodzi crushpen (antibody prevalence of 38.5%), trypanosomosis was virtually absent in cattle sampled along the lakeshore in the Central Region (Salima District). Trypanosomosis was also absent in Ntcheu and Dowa Districts.

Table 3.4.7: Average PCV (%), parasitological and anti-trypanosomal antibody prevalence (%) in each of the districts surveyed in the Central Region of Malawi.

District	Number of herds	Average PCV (% ± 1 s.e.)	Parasitology		Serology	
			Number positive (%)	Number sampled	Number positive (%)	Number sampled
Dedza	4	31.2 ± 0.3	3 (1.3)	240	55 (28.6)	192
Dowa	1	32.9 ± 0.6	0 (0)	60	0 (0)	55
Mchinji	2	28.6 ± 0.5	1 (0.8)	120	20 (20.8)	96
Kasungu	13	31.6 ± 0.2	17 (2.6)	644	43 (8.5)	503
Lilongwe	7	31.0 ± 0.3	1 (0.2)	420	31 (11.1)	280
Nkhotakota	8	31.0 ± 0.3	25 (6.0)	414	29 (7.9)	368
Ntcheu	5	31.9 ± 0.3	0 (0)	300	4 (1.5)	257
Ntchisi	2	31.1 ± 0.5	1 (0.8)	120	3 (3.9)	76
Salima	16	32.3 ± 0.2	0 (0)	960	49 (6.2)	788

Figure 3.4.4: Herd average PCV, proportion of animals, parasitological and serological prevalence of bovine trypanosomosis in the Central Region of Malawi

LEGEND

Tarred Road........................
Other Road ....
Railway..............................
Lake ....
Pan, Swamp ....

National Land ie. Park,
Game & Forest Reserve
International Boundary
Provincial Boundary ....
Sample Site, ... O

Average per area....................

Average packed cell volume (%) ....
Animals PCV≤24%(%) ....
Parasitological prevalence (%)...........
Serological prevalence........................

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(iii) Southern Region

A major trypanosomosis focus in the Southern Region is the area east of Liwonde National Park and Liwonde Forest Reserve (Table 3.4.8 and Fig. 3.4.5). The prevalence of anti-trypanosomal antibodies was high in cattle sampled at Mposa (97.4%) and Namasalima (58.8%) crushpens (Machinga and Zomba Districts). Trypanosomal infections (7 *T. congolense* (12.7%)) were detected only in samples collected from animals at Mposa crushpen (Zomba District). The prevalence of cattle with trypanosomal infections and anti-trypanosomal antibodies was also high closer to the Mozambican border to the east (Fig. 3.4.5). Thirty-three percent of the animals sampled at Mikoko crushpen, north of Lake Chilwa were infected with trypanosomes (all *T. congolense*). The anti-trypanosomal antibody prevalence remained high (51.3 ± 24.5%) in animals sampled south of Lake Chilwa in the stretch along the Mozambican border (Mulanje District) (Fig. 3.4.5). However, trypanosomal infections were not detected. Phalula was the only crushpen west of the Shire River in Zomba and Machinga Districts that had a high proportion of cattle with anti-trypanosomal antibodies (91.1%). In Chikwawa District the parasitological prevalence of trypanosomosis was high in the herd sampled at Shire Valley Ranch (20 *T. congolense* (33.3%)) and Mwananjovu crushpen (10 *T. congolense* (16.7%)) (Fig. 3.4.5). Except for cattle sampled at these two sites, the prevalence of anti-trypanosomal antibodies was low (on average 3.7 ± 0.9%) in Chikwawa District. Trypanosomal infections (4 *T. congolense* (6.7%)) were also detected in cattle sampled at Phokera crushpen at the edge of Mwabvi Game Reserve (Nsanje District). In the southern part of Nsanje District, trypanosomosis was prevalent. A total of 25 *T. congolense* infections (13.9%) were detected in cattle sampled at Benje, Lulwe and Thundu crushpens (Fig. 3.4.5). Trypanosomosis was absent in Mwanza, Blantyre and Thyolo Districts.

Table 3.4.8: Average PCV (%), parasitological and anti-trypanosomal antibody prevalence (%) in each of the districts surveyed in the Southern Region of Malawi.

District	Number of herds	Average PCV (% ± 1 s.e.)	Parasitology		Serology	
			Number positive (%)	Number sampled	Number positive (%)	Number sampled
Blantyre	2	31.6 ± 0.4	0 (0)	110	3 (7.7)	39
Chikwawa	13	32.3 ± 5.7	30 (3.8)	780	38 (8.5)	449
Machinga	8	28.2 ± 0.3	44 (9.4)	470	50 (21.8)	229
Mangochi	10	31.9 ± 0.2	6 (1.0)	587	40 (8.6)	466
Mulanje	5	31.4 ± 0.3	0 (0)	281	62 (26.6)	233
Mwanza	6	30.6 ± 0.2	0 (0)	360	4 (2.7)	149
Nsanje	7	32.0 ± 0.3	29 (6.9)	420	55 (15.6)	352
Thyolo	2	33.8 ± 0.6	0 (0)	88	1 (1.1)	88
Zomba	4	30.7 ± 0.3	7 (3.0)	235	33 (22.4)	147

Figure 3.4.5: Herd average PCV, proportion of [anaemic] animals, parasitological and serological prevalence of bovine trypanosomiasis in the Southern Region of Malawi.

3.4.4 Discussion and conclusions

3.4.4.1 Parasitological and serological prevalence of bovine trypanosomosis

In all areas surveyed, most trypanosomal infections were *T. congolense*. This is in accordance with observations made in most countries of southern Africa. The majority of the animals in which trypanosomal infections was diagnosed parasitologically had anti-trypanosomal antibodies. Hence, the diagnostic sensitivity of the antibody-detection ELISA was high but differed between trypanosome species. The sensitivity was highest for *T. congolense* infections and was similar to the original sensitivity, at a cut-off of 28%, when known positive samples collected in Zambia were used (Hopkins *et al.*, 1998). The sensitivity of the antibody ELISA to detect *T. vivax* infections, on the other hand, appeared to be much lower. Although *T. vivax* infections only constituted a small proportion of all trypanosomal infections, this observation requires further investigation. The prevalence of anti-trypanosomal antibodies was much higher than the parasitological prevalence of infection. Only a small proportion of the seropositive animals (10.9%) was infected with trypanosomes. This is not surprising considering the low sensitivity of parasitological diagnostic methods for trypanosomosis (Paris *et al.*, 1982) and the persistence of anti-trypanosomal antibodies in the absence of infection (section 3.2.3). Hence, some of the seropositive but parasitologically negative animals were likely to be infected with trypanosomes (false negatives) whereas others may have been infected with trypanosomes but the infection had been cured. It is difficult to allocate a seropositive animal to one of those two categories. However, on the basis of the PCV of a seropositive animal, assumptions can be made on its infection status. One of the most typical signs of bovine trypanosomosis is the development of anaemia which is best measured by determining the PCV (Stephen, 1986). Hence, the significantly lower PCV of cattle infected with trypanosomes and the significant correlation between parasitological prevalence of trypanosomosis and herd average PCV. The significantly lower PCV of parasitologically negative and seropositive animals compared to parasitologically negative and seronegative animals does indeed suggest that a proportion of the seropositive animals was infected at the time of sampling or had recently been infected with trypanosomes, which caused a reduction in their PCVs. Moreover, the correlation between the PCV and the percentage positivity of

these parasitologically negative but seropositive animals suggests that the false negative animals are most likely those with the highest antibody titre. Although, on the basis of these results, no statements can be made on the specificity of the antibody ELISA, the relationships described above do indicate that the antibody detection ELISA does detect false negative animals and can be used as a useful supplementary diagnostic test to improve the accuracy of trypanosomosis surveys.
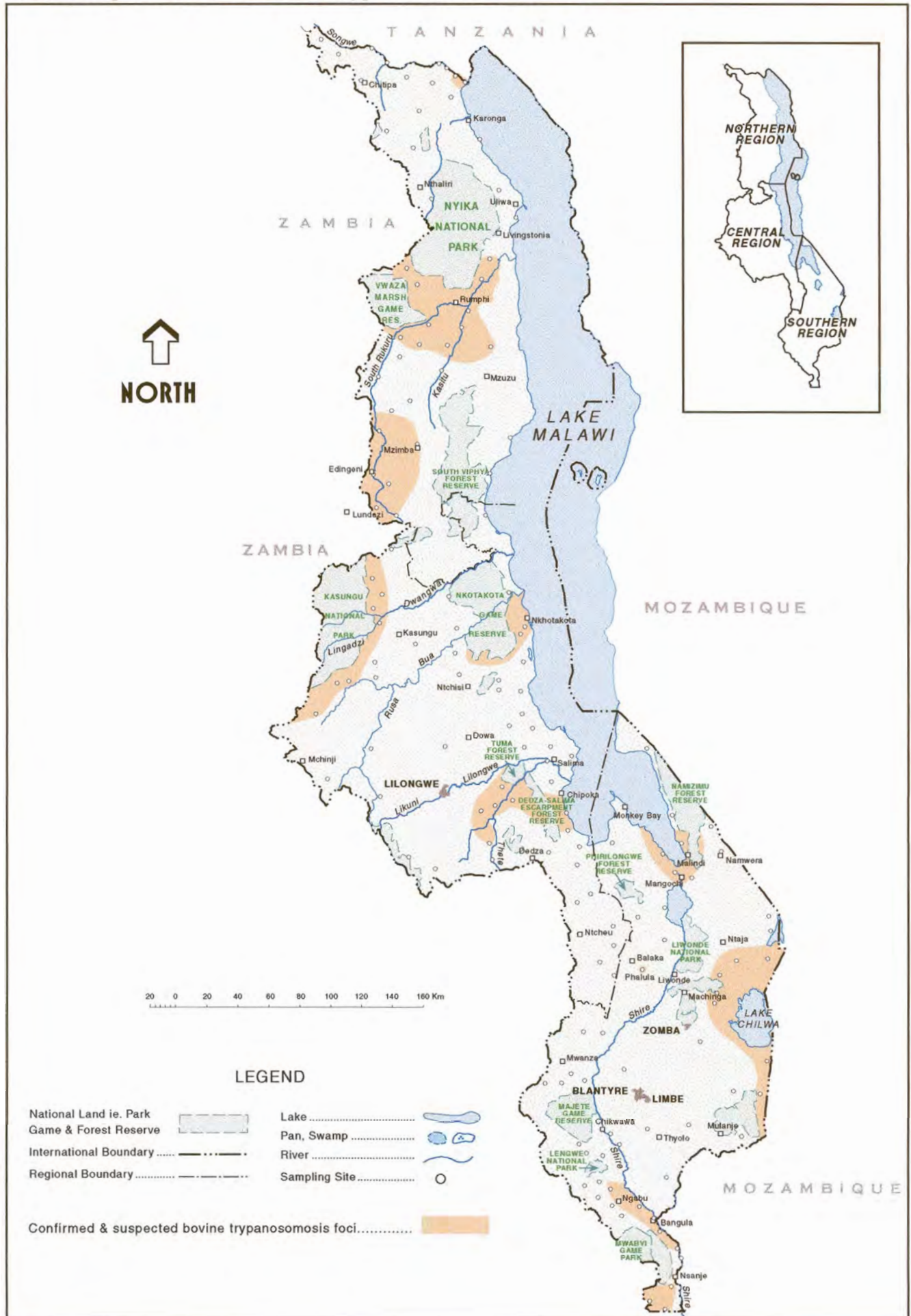
3.4.4.2 Distribution and epidemiology of bovine trypanosomosis in Malawi

The main factor affecting the distribution of tsetse and, hence, bovine trypanosomosis in Malawi is the expansion of the human population and concomitant destruction of the vegetation. As a result, *G. m. morsitans* and *G. pallidipes* primarily occupy national parks, game reserves and forest reserves where habitat is suitable and game animals which constitute the major food source occur. *Glossina brevipalpis* is mainly found along the shore of Lake Malawi or along rivers, often in small patches of dense vegetation (Sanderson, 1910; Mitchell and Steele, 1956). Parasitologically positive herds were located in the vicinity of known tsetse foci within Malawi or adjacent to tsetse-infested areas in neighbouring countries. Bovine trypanosomosis was diagnosed in areas surrounding the Kasungu National Park, the Nkhotakota Game Reserve, the Vwaza Game Reserve, the Liwonde National Park, the Lengwe National Park and the Tuma Forest Reserve (Fig. 3.4.6). Despite the abundance of tsetse in most of these foci, the parasitological prevalence of bovine trypanosomosis was generally low. This is not surprising considering the abundance of suitable wild hosts in the game areas and the restricted tsetse/cattle interface along the edges of the tsetse-infested areas. Moreover, the odour-baited, insecticide-treated, target barriers (Hargrove, 1993) along the edge of Kasungu National Park and the Nkhotakota Game Reserve have reduced substantially the prevalence of bovine trypanosomosis in herds surrounding both game areas.

The distribution of parasitologically positive herds correlated well with the picture obtained from the 1987-89 National Trypanosomosis Survey (Davison, 1990). The most northern trypanosomosis focus (Mwangurukuru crushpen) is still attributed to the presence of *G. brevipalpis*. This tsetse pocket was first identified in 1909

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(Sanderson, 1910; Lamborn, 1915) and has reduced substantially in size since the last surveys (Mitchell and Steele, 1956; Davison, 1990). Hitherto, little was known of the distribution and the epidemiological importance of *G. brevipalpis* in Malawi. Because of ecological separation of its habitat and the grazing areas of cattle and the high proportion of feeds on hippopotamus (*Hippopotamus amphibius*), *G. brevipalpis* has rarely been implicated as an important vector of bovine trypanosomosis (Weitz, 1963). Nevertheless, *G. brevipalpis* has been observed feeding on cattle (Sanderson, 1910) and tsetse species of the *fusca*-group are good vectors of cattle trypanosomosis (Leak *et al.*, 1991). Moreover, it occurs outside the protected areas (game parks and forest reserves) and the gradual clearing of land for agriculture may result in an increased contact between cattle and this tsetse species. In some areas of the KwaZulu-Natal Province of South Africa, for example, *G. brevipalpis* is considered to be the main source of infection for cattle (Kappmeier *et al.*, 1998). The importance of *G. brevipalpis* in the epidemiology of bovine trypanosomosis in Malawi may, therefore, be underrated and could increase in the future. Since the distribution of *G. brevipalpis* is not well defined, insecticide treatments of cattle may be an effective means of controlling this tsetse species. The Vwaza Game Reserve was the main tsetse-infested area (*G. m. morsitans* and *G. pallidipes*) in the Northern Region of Malawi and determined the distribution of bovine trypanosomosis in the Vwaza area. The distribution of *G. m. morsitans* and *G. pallidipes* was, however, not restricted to the boundaries of the Game Reserve (Mitchell and Steele, 1956; Davison, 1990). This is indicated by the distribution pattern of cattle with anti-trypanosomal antibodies. The movement of tsetse away from their prime focus is common in Malawi and was first described by Shircore (1914). He found that, in the dry season, tsetse were confined to the most favourable habitat in their prime foci. However, during the rainy season when climatic conditions are favourable tsetse may move into surrounding areas. The extent of dispersion during the rainy season will depend largely on availability of suitable habitat and host density. The South Rukuru River provides an ideal conduit for such seasonal movements of tsetse in the Vwaza area, which probably explains the widespread distribution of cattle with anti-trypanosomal antibodies in the Rumphi District. Similar seasonal changes in the distribution of tsetse were observed by Davison (1990) in the Chief Chulu area along the fringe of the Kasungu National

Figure 3.4.6: Confirmed (trypanoso... suspected (anti-trypanosomal antibodies present) foci of bovine trypanosomosis in Malawi.

LEGEND

National Land ie. Park Game & Forest Reserve	Lake
International Boundary	Pan, Swamp
Regional Boundary	River
	Sampling Site

Confirmed & suspected bovine trypanosomosis foci

Park. These changes cause seasonal variations in the level of tsetse challenge to cattle that are often highly susceptible. The reasons for and the extent of the seasonal fly movements are not fully known but they appear to be an important part of the epidemiology of bovine trypanosomosis in Malawi as they were in the original situation in Zululand (South Africa) and very likely in parts of Zimbabwe, especially where *G. pallidipes* is present. They explain the presence of cattle with anti-trypanosomal antibodies in areas far removed from the original tsetse focus and the occurrence of bovine trypanosomosis epidemics such as those observed between 1982-85.

The presence of cattle with anti-trypanosomal antibodies south west of Mzimba is attributed to the Lundazi tsetse-belt (*G. m. morsitans*) in eastern Zambia which extends into Malawi.

In the Central Region, the Kasungu National Park and the Nkhotakota Game Reserve were the main tsetse foci (mainly *G. m. morsitans*). Tsetse density in both wildlife areas was high (Davison, 1990) and the prevalence of human sleeping sickness and bovine trypanosomosis reached epidemic proportions a decade ago (unpublished reports. Department of Animal Health and Industry). This problem was effectively alleviated by the deployment of a 6 km-wide odour-baited, insecticide-treated, target barrier (Hargrove, 1993) along the eastern edge of Kasungu National Park and the southern part (south of Bua River) of the Nkhotakota Game Reserve (RTTCP, 1996). Both target barriers have been very effective in reducing challenge. This is clearly reflected in the low parasitological prevalence of bovine trypanosomosis and, especially, the low prevalence of anti-trypanosomal antibodies in the areas surrounding these two tsetse foci. Moreover, whereas bovine trypanosomosis used to be prevalent throughout the area between the two game parks (Davison, 1990) it is now confined to the immediate vicinity (approximately 10-15 km) of both tsetse-infested zones. The effect of the target barriers is twofold. First, they effectively reduce contact between tsetse and cattle at the edge of the game parks and second, they almost entirely prevent the seasonal movement of tsetse. The absence of a target

barrier in the southern section of Kasungu National Park explains the southward spread of the distribution of bovine trypanosomosis.

Glossina m. morsitans has been recorded in the Dedza-Salima Escarpment Forest Reserve and the Tuma Forest Reserve (Mitchell and Steele, 1956). However, during the 1987-89 survey, flies were only found in the Tuma Forest Reserve. According to these survey results challenge occured in both areas. A distinct reduction in the distribution of tsetse and, hence, trypanosomosis was observed in the Salima area. Whereas *G. m. morsitans* used to infest most of the valley floor north of Salima (Shircore, 1914), bovine trypanosomosis was virtually absent at the time of the present survey. Trypanosomosis was also completely absent in cattle sampled near the Phirilongwe Forest Reserve focus. Although this forest reserve was infested with *G. m. morsitans* during the 1987-89 survey, progressive infiltration of settlements may have resulted in the eradication of the fly from this focus during the past decade. The presence of *G. m. morsitans* in the Mamizumu and the Mangochi Forest Reserves (Mitchell and Steele, 1956; Davison, 1990) explains the prevalence of bovine trypanosomosis along the southern part of Lake Malawi. The Shire River formed the boundary between a trypanosomosis-free and trypanosomosis-infested area in the northern part of the Southern Region. With the exception of the high prevalence of anti-trypanosomal antibodies in cattle sampled at Phalula, bovine trypanosomosis was absent in the area west of the river. Since Phalula is located close to the bridge across the Shire River, the high prevalence of cattle with antibodies can be attributed to the movement of cattle with anti-trypanosomal antibodies from the trypanosomosis-infested, eastern area into the trypanosomosis-free, western area.

The main tsetse focus (*G. m. morsitans*) east of the Shire River was the Liwonde National Park. The bovine trypanosomosis cases east of the National Park could have been due to challenge by tsetse from the Liwonde National Park although few cattle graze in the immediate vicinity of this tsetse focus. It is more plausible that the trypanosomal infections east of Liwonde National Park and north, west and south of Lake Chilwa were a result of fly-belts from Mozambique's Mecanhelas and Milange Districts extending into Malawi.

Despite the presence of *G. pallidipes* in Majete Game Reserve (Davison, 1990), the parasitological and serological prevalence of bovine trypanosomosis in herds sampled in areas surrounding the Game Reserve was low. This is in contrast with the 1987-89 trypanosomosis survey results (Davison, 1990). Nevertheless, the present results are in accordance with the limited distribution of tsetse in the Game Reserve as observed by Davison (1990). Similarly, cattle seem to have been challenged very little by the tsetse (*G. pallidipes* and *G. m. morsitans*) present in the Lengwe National Park. Moreover, the tsetse pockets south of the National Park and the bovine trypanosomosis focus in this area (Davison, 1990) seem to have disappeared. In view of significant reduction in the distribution of bovine trypanosomosis in areas surrounding the Lengwe National Park, the trypanosomal infections in cattle north and east of Ngabu (Shire Valley Ranch an Mwananjovu crushpen) cannot be attributed to challenge by tsetse from the Lengwe National Park. However, both sampling sites are located close to the Elephant Marsh. Little is known of the current tsetse situation in the Elephant Marsh but *G. brevipalpis* has been reported (Austen, 1903; Lamborn, 1915; Potts, 1954). Further investigations are required. The trypanosomosis cases detected in the most southern part of the country can be attributed to the presence of tsetse (*G. pallidipes* and *G. m. morsitans*) in the Mwabvi Game Reserve, the Matandwe Forest Reserve and challenge by tsetse from neighbouring Mozambique (Morrumbala and Mutarara Districts).

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

3.5.1 Introduction

In Namibia, tsetse-transmitted trypanosomosis or "nagana" is restricted to the Caprivi District. The distribution of tsetse (*G. m. 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 *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.

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.

3.5.2 Materials and methods

3.5.2.1 Sampling area

The Eastern Caprivi lies to the east of the Kwando River (Fig. 3.5.1) in the Caprivi District (Namibia). It is over 11 600 km^2 in extent and has a population of over 122 000 head of cattle. The only tsetse species occurring is *G. m. centralis*.

Between August 1995 and June 1997, a survey of bovine trypanosomosis was conducted at 33 sampling sites (Fig. 3.5.1).

To facilitate the interpretation of the survey results, the sampling area was subdivided into survey areas. Sampling sites were categorised according to the grazing areas of the cattle and allocated to one of the survey areas. Four survey areas were identified (Table 3.5.1):

Table 3.5.1: Survey areas and sampling sites in Eastern Caprivi, Namibia.

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	Izwili		Saujuo
	Kalubi		Nongozi
	Kongola		Mbambazi
	Ngonga		Lizauli
	Singalamwe		Samudondo
			Malengalenga

Figure 3.5.1: Map of the survey area and location of the sampling sites in the Eastern Caprivi, Namibia.

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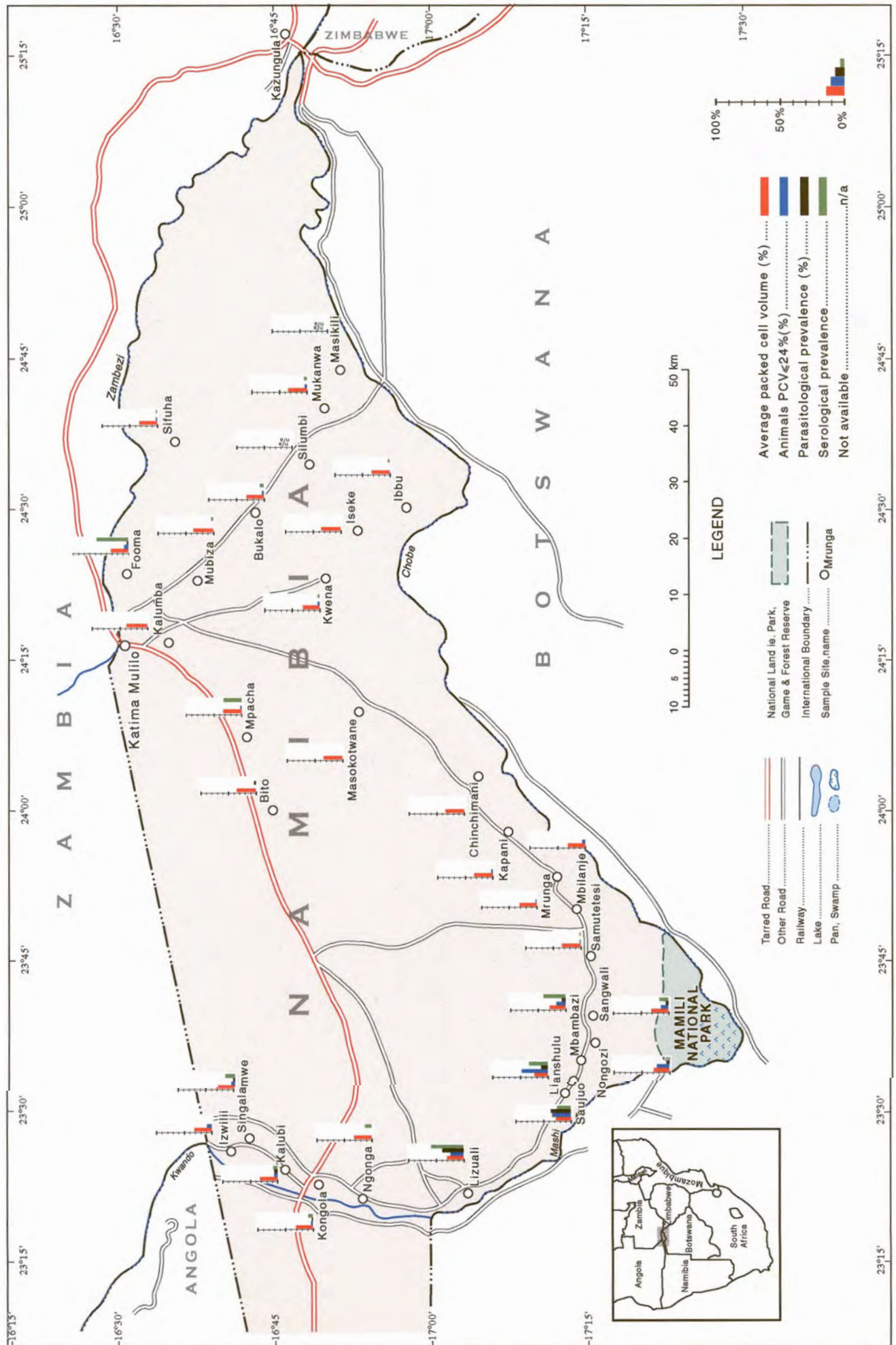
- the "Katima Mulilo area" (cattle grazing along the Zambezi and immediately south of Katima Mulilo).
- the "Kwando area" (cattle grazing along the Kwando River, north of Ngonga),
- the "Mamili area" (cattle grazing along the Kwando/Mashi River north of the Mamili National Park) and
- the "Linyanti/Chobe 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. 3.5.1). Trypanocides, mainly diminazene aceturate (Berenil®, Hoechst), were used frequently in cattle herds in the Mamili survey area.

3.5.2.2 Sampling size

A total of 1 481 adult cattle were examined. A cross-sectional sampling method was applied (Section 3.4.2.1).

3.5.2.3 Sampling method

Direct parasitological serological (anti-trypanosomal antibody detection ELISA) diagnostic tests were used (Section 3.3.2.2).

3.5.3 Results

3.5.3.1 Parasitological prevalence of bovine trypanosomosis

A total of 1 481 samples were examined. Tsetse-transmitted trypanosomes were detected in 66 animals (4.5%) sampled at 14 of the 33 sampling sites (Table 3.5.2).

All infections were detected on buffy coat and confirmed on thick and thin smears. The parasitological prevalence of *T. 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*.

Table 3.5.2: Sample size, number of animals with trypanosomal infections and average PCV (% ± 1 s.e.) of herds sampled at various sampling sites in the different survey areas, Eastern Caprivi, Namibia.

Survey area	Sample site	Sample size	T. vivax	T. congolense	T. brucei	mixed (Tc/Tv)	Average PCV (%) (± 1 s.e.)
Katima	Mpacha	55	0	0	0	0	33.4 ± 0.5
Mulilo	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/	Sangwali	60	3	0	0	0	31.1 ± 0.7
Chobe	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	-

The mean parasitological prevalence was low in the Katima Mulilo, Kwando and Linyanti/Chobe survey area (Table 3.5.3). A significantly higher mean parasitological prevalence was found in the Mamili survey area (Table 3.5.3).

3.5.3.2 Packed cell volume

Table 3.5.2 summarizes the average PCV (± 1 s.e.) of herds sampled in each of the survey areas. Packed cell volume profiles for each of the four survey areas are presented in Figure 3.5.2.

The mean PCVs were significantly different between all survey areas (P<0.001). The percentage of anaemic animals 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).

3.5.3.3 Prevalence of anti-trypanosomal antibodies

A total of 1 196 blood spots were screened for anti-trypanosomal antibodies (Table 3.5.4). Only 115 samples (9.6%) were serologically positive.

Table 3.5.3: Number of samples, average serological prevalence, parasitological prevalence of trypanosomosis and average packed cell volume of herds sampled in each of the survey areas, Eastern Caprivi, Namibia.

Survey area	Number of samples	Average parasitological prevalence (%) (± 1 s.e.)	Number of samples	Average serological prevalence (%) (± 1 s.e.)	Number of samples	Average PCV (%) (± 1 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
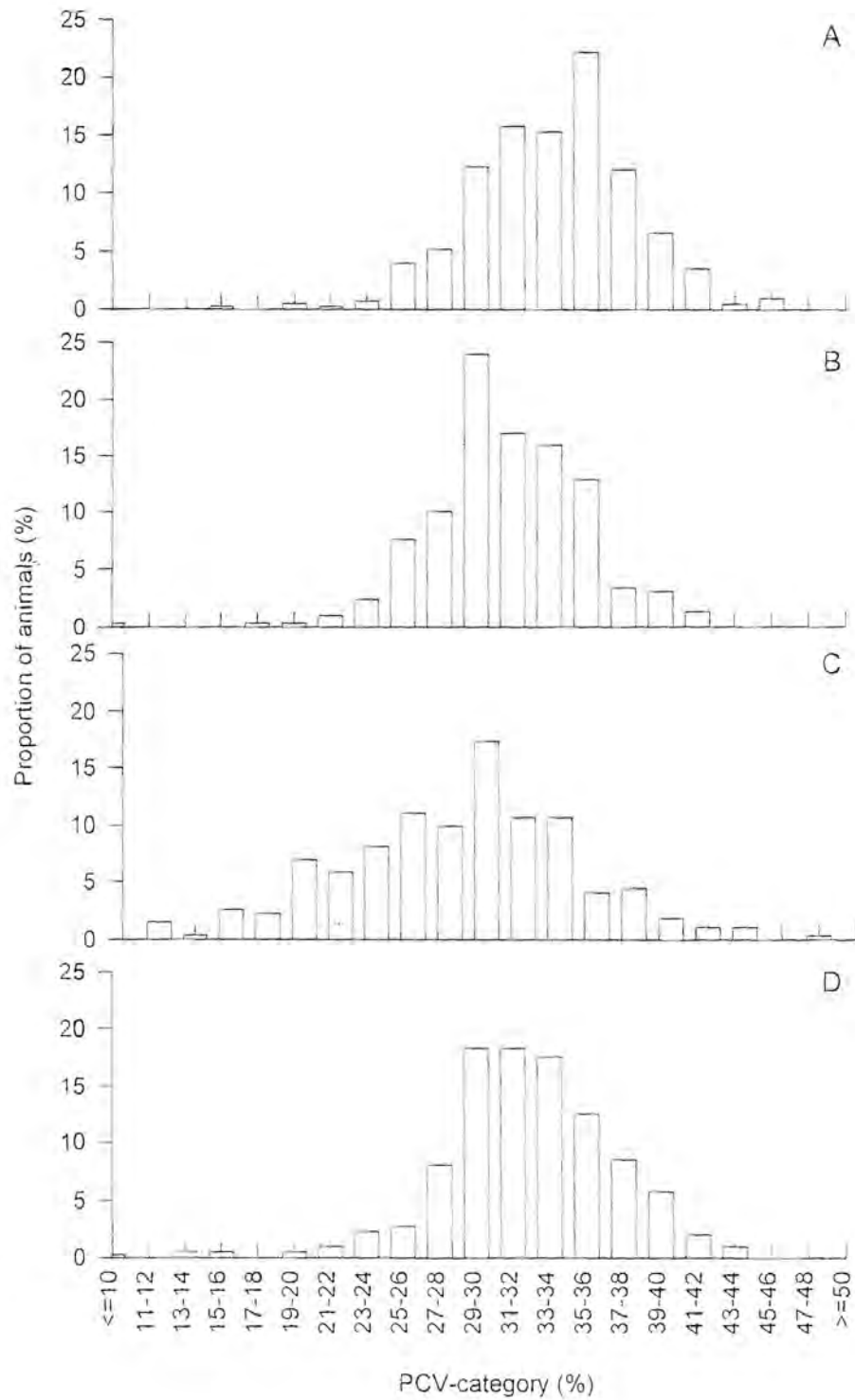
Figure 3.5.2: Comparison of PCV profiles of herds sampled in the Katima Mulilo (A), Kwando (B), Mamili (C) and Linyanti/Chobe (D) survey areas, Eastern Caprivi, Namibia.

Table 3.5.4: Sample size, number of positives, average percentage positivity (% ± 1 s.e.) and serological prevalence of samples collected at various sampling sites in the different survey areas, Eastern Caprivi, Namibia.

Survey area	Sample site	Sample size	Number serological positive	Average Percentage Positivity (± 1 s.e.)	Serological Prevalence (%)
Katima Mulilo	Mpacha	49	14	21.3 ± 1.3	32.3
	Bito	27	0	16.5 ± 0.7	0
	Kalumba	10	0	16.6 ± 1.5	0
	Kwena	30	1	18.4 ± 0.8	3.7
	Masokotwani	47	0	16.6 ± 0.5	0
	Mubiza	39	2	16.4 ± 0.9	3.7
	Fooma	40	2	28.6 ± 1.9	56.5
	Bukalo	31	2	18.6 ± 0.7	7.3
	Iseke	32	0	18.7 ± 0.7	0
	Sifuha	46	1	17.9 ± 0.0	2.5
	Silumbi	9	0	15.2 ± 1.8	0
Kwando	Kalubi	59	0	15.5 ± 2.0	7.8
	Kongola	57	4	21.5 ± 1.1	8.3
	Izwilii	59	0	15.5 ± 0.5	0
	Singalamwe	47	7	20.7 ± 1.2	17.5
	Ngonga	50	5	15.4 ± 1.1	11.8
Mamili	Mbambazi	53	18	22.2 ± 1.7	40.0
	Samudono	59	0	13.3 ± 0.7	0
	Lizauli	52	26	27.7 ± 1.7	58.8
	Lianshulu	52	15	24.5 ± 1.9	33.9
	Saujuo	33	7	21.2 ± 2.6	25.0
Linyanti/ Chobe	Sangwali	57	8	15.7 ± 1.5	16.5
	Samutetesi	54	1	12.1 ± 0.5	2.2
	Mbilanje	57	0	13.5 ± 0.5	0
	Kapani	37	0	8.4 ± 0.8	0
	Mrunga	57	0	14.6 ± 0.5	0
	Chinchimani	20	0	15.6 ± 0.7	0
	Mukanwa	23	1	17.9 ± 0.8	4.8
	Ibbu	33	1	20.2 ± 0.6	3.3
	Masikili	13	0	19.2 ± 0.9	0

Anti-trypanosomal antibodies were detected in herds sampled at 18 out of the total of 30 sample sites where blood spots were collected (Table 3.5.4). The serological prevalence varied considerably between locations (Table 3.5.4). 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 prevalence of trypanosomosis at the various sampling sites.

Anti-trypanosomal antibody titres were found in cattle from nine sampling sites where animals were parasitologically negative. The average serological prevalence in the parasitologically negative herds was 7.9% compared to 29.4% in herds that were parasitologically positive. The average serological prevalence in each of the survey areas is summarised in Table 3.5.4.

The percentage of anaemic animals at a sampling site was significantly correlated ($r = 0.59$, $P<0.01$) with the serological prevalence at that site.

3.5.4 Discussion and conclusions

3.5.4.1 Parasitological and serological prevalence of bovine trypanosomosis

According to the parasitological and serological prevalences, tsetse-transmitted trypanosomal infections in the Eastern Caprivi are confined to the Kwando River drainage and the 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 5 km-wide Mamili target barrier cannot 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, 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. Unfortunately, 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). From 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 other biting flies in transmitting *T. vivax* to cattle in tsetse-infested areas remains an unresolved issue (D'Amico *et al.*, 1996). Second, there 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 than 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 would, nevertheless, permit 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.

3.5.4.2 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. 3.5.2). 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.

3.5.4.3 Interpretation of serological data

In contrast with the parasitological methods, the serological test used in this survey had 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 *et al.*, 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 adequate sensitivity (Paris *et al.*, 1982). Although antibody detection tests cannot form the basis of identifying infected animals (Nantulya, 1990), a decline in antibody prevalence can be used to assess the impact of tsetse control operations 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 and Mudenge, 1997).

An important reason for conducting this serological survey was to determine whether the population or herd had been exposed to trypanosomosis. In this respect, areas of particular interest were those where disease prevalence was too low to detect parasites by current parasitological diagnostic methods or where tsetse could not be captured. In such cases, the determination of the anti-trypanosomal antibody prevalence of a herd made it possible to distinguish with a high degree of confidence between low challenge and no challenge. One such example was 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 have developed antibody response at the time of sampling, 90 % of the parasitologically positive herds were also serologically positive. Moreover, herd seroprevalence was positively correlated to parasitological prevalence and the percentage of anaemic animals. These findings indicate that, on a herd basis, the prevalence of anti-trypanosomal antibodies may be used to assess the infection status or extent of disease.

3.6 An evaluation of the usefulness of the anti-trypanosomal antibody detection ELISA as a tool for monitoring the effectiveness of tsetse control operations in Zimbabwe

3.6.1 Introduction

In Zimbabwe, tsetse control has a long history. During the past 65 years, large portions of land have been cleared from tsetse through concerted effort of Zimbabwe's Tsetse and Trypanosomiasis Control Branch (T&TCB). Between 1986 and 1998, for example, approximately 20 400 km^2 of area was cleared of tsetse using a variety of control methods (Lovemore, 1999). In most areas, odour-baited target barriers (in some areas supported by insecticide-treated cattle) have been put in place to prevent tsetse from re-invading cleared areas. In other areas, where the risk of reinvasion by tsetse is low, artificial barriers are absent. The effectiveness of these barriers or the absence of tsetse in areas not protected by barriers is monitored through continuous tsetse and irregular trypanosomosis surveillance. Such surveillance exercises are time consuming and expensive. Moreover, due to the low sensitivity of the currently available tsetse and trypanosomosis surveillance methods (Paris et al., 1982; Hargrove, 1980a), low-density tsetse populations and areas where the prevalence of trypanosomosis is low may be missed and, hence, regarded erroneously as tsetse or disease-free. The recently improved anti-trypanosomal antibody detection enzyme-linked immunosorbent assay (antibody ELISA) (Hopkins et al., 1998) could be used as an additional tool for monitoring the effectiveness of tsetse control operations. The assay has high diagnostic sensitivity and specificity in detecting anti-trypanosomal antibodies in cattle. Moreover, the assay detects antibodies against current and past trypanosomal infections. Finally, because of the use of filter papers to collect blood samples, sample collection and storage is simplified.

To determine the usefulness of the anti-trypanosomal antibody detection ELISA as an additional tool to monitor the effectiveness of tsetse control operations, a trypanosomosis survey was conducted along Zimbabwe's tsetse front. The prevalences of anti-trypanosomal antibodies in cattle were determined at each of the sampling sites and were compared with the parasitological prevalence of

trypanosomal infections and current and historical data on the distribution and density of tsetse. Conclusions were drawn on the current trypanosomosis situation in the country and the usefulness of the antibody ELISA as a monitoring tool is discussed.

3.6.2 Materials and methods

3.6.2.1 Sampling sites and sample selection

Between January 1998 and September 1999, a total of 3 988 adult cattle were examined at 62 sampling sites in the southeastern, eastern/northeastern, northern and western regions of Zimbabwe (Fig. 3.6.1). Since the aim of the survey was to monitor the current bovine trypanosomosis situation, sampling was restricted to areas, supposedly cleared of tsetse, adjacent to the tsetse invasion front or along barriers to prevent re-invasion.

A cross-sectional sampling method was applied (Section 3.4.2.1).

Figure 3.6.1: Location of sampling sites in Zimbabwe.

LEGEND

— Trunk Road
— Other Road
- - - Target barrier
River
○ Cholo Sample site, name

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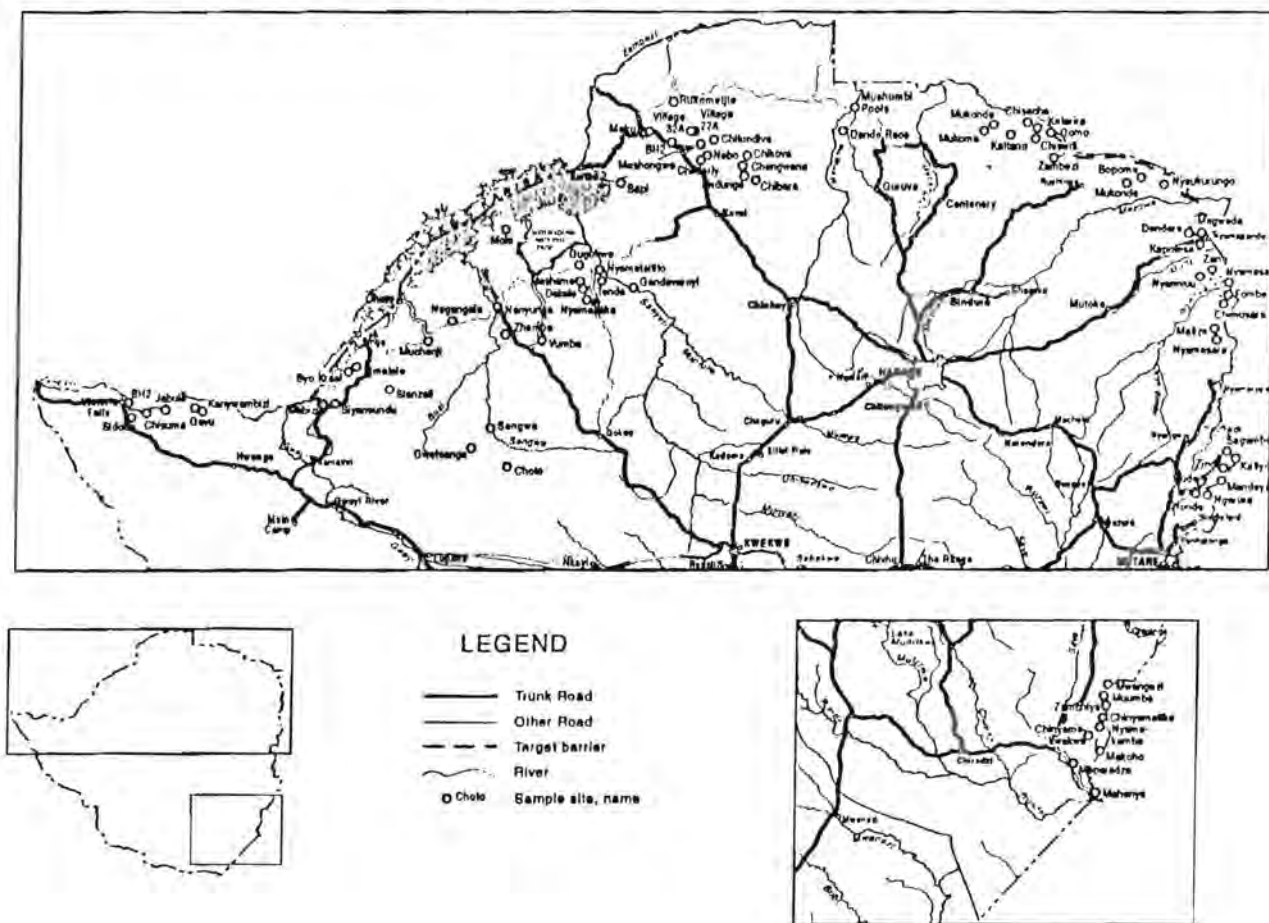
3.6.2.2 Survey areas

(i) Chipinge area

Tsetse were eradicated from the south-east lowveld as the result of a large-scale joint ground spraying operation between the governments of Mozambique, South Africa and Zimbabwe (then Rhodesia) (Robertson and Kluge, 1968). Between 1962 and 1981, about 5 700 km^2 of the southeastern lowveld was cleared of *G. m. morsitans* and *G. pallidipes* (Robertson and Kluge, 1968). Furthermore, because of the spraying campaigns in Mozambique the tsetse front was pushed up to Massangena about 60 km east of the Zimbabwe border. Despite the absence of measures to contain the westerly advance of the fly front, no cases of bovine trypanosomosis have been diagnosed in the southeastern lowveld since the spraying operation. In 1997, however, two tsetse flies were captured at Mavué along the Zimbabwe/Mozambique border immediately south of the Save River (RTTCP, 1999a). Moreover, the prevalence of cattle with anti-trypanosomal antibodies sampled at Mavué was high (59%). Because of the potential threat of reinvasion of tsetse into Zimbabwe, intensive tsetse surveillance was initiated in 1998 in the Chipinge area. No tsetse were captured. Nevertheless, the threat of tsetse reinvading the southeastern lowveld is present.

(ii) Honde Valley

In the Honde Valley bovine trypanosomosis was recorded in 1959 (Thakersi, 1992). In 1992 a major outbreak of the disease occurred. This outbreak was attributed to the spread of *G. pallidipes*, *G. m. morsitans* and *G. austeni* from neighbouring Mozambique (Thakersi, 1992) and was controlled by dipping of cattle in 0.00375% deltamethrin (Decatix®, Coopers) at two-weekly intervals. Because of the significant improvement of the trypanosomosis situation insecticide treatments of cattle ceased at the end of 1996. Since that period little information is available on the tsetse and trypanosomosis situation in the Honde Valley.

(iii) Eastern/northeastern region

In Centenary, Mount Darwin, Rushinga, Mudzi and Nyanga Districts, tsetse are restricted to the international border with Mozambique. An odour-baited, insecticide-treated, targets barrier is present in most of the border areas. It was

removed between Musengezi River and Chigango. Because of intense invasion pressure of tsetse (*G. m. morsitans* and *G. pallidipes*) between Nyamapanda and the Ruenya River in Mudzi District, the target barrier was supplemented by an additional target operation, on average 10 km wide, with a target density of 4 targets/km². In Guruve District, target operations supplemented by deltamethrin-treated cattle are active in the Dande Communal Land and the Dande Safari Area. Tsetse surveillance and survey results are used to evaluate the effectiveness of the eastern/north eastern border tsetse control operation. Trypanosomosis surveys are conducted at irregular intervals by the Department of Veterinary Services.

(iv) Northern region

Between the eastern edge of Lake Kariba and the Manyame River, tsetse-cleared areas are protected from re-invasion by active odour-baited, insecticide-treated, target operations of varying width (Lovemore, 1999). The effectiveness of this target barrier is evaluated as in the eastern/north eastern border region.

(v) Western region

With the exception of the area covered by the Matusadona National Park, large-scale aerial and ground spraying operations were conducted between 1982 and 1987 with the aim to clear the western region of tsetse (Hursey and Allsopp, 1984; Allsopp and Hursey, 1986; Lovemore, 1990). Despite all these efforts, tsetse (*G. pallidipes*) were still being captured along the drainage of the Busi and Sengwa Rivers and in the communal land south of the Chirisa Safari Area (Sengwa Gorge) (Lovemore, 1990). As a result of these catches and the detection of trypanosomal infections in cattle sampled in areas surrounding the Chirisa Safari Area, a target operation and dipping of cattle in deltamethrin was initiated at the end of 1988. At the same time, a 10 months' trypanosomosis surveillance exercise was initiated at six diptanks (including Choto and Gwetsanga) until July 1989 (RTTCP, 1989). Following these measures no tsetse have been caught since December 1989, and the last trypanosomal infection in the sentinel cattle was detected in March 1989 (RTTCP, 1989).

Tsetse disappeared from the banks of the Zambezi between Victory Falls and the Gwayi River after the rinderpest outbreak at the end of the 19th century (Jack, 1914). However, in 1972 an isolated outbreak of bovine trypanosomosis was reported at Katchetechete (Lovemore and Napier Bax, 1972). Since that period, no bovine trypanosomosis outbreaks have been reported from the area. In 1990, a trypanosomosis survey conducted along the Zambezi River east of Victoria Falls could not detect trypanosomal infections (RTTCP, 1990). However, trypanosomosis surveys in Zambia revealed a southerly advance from the tsetse (*G. m. centralis*) infestation centred on the Kafue National Park (RTTCP, 1991). The threat to Zimbabwe of this southerly advance of tsetse is present.

3.6.2.3 Diagnostic methods

Direct parasitological and serological (anti-trypanosomal antibody detection ELISA) diagnostic tests were used (Section 3.3.2.2).
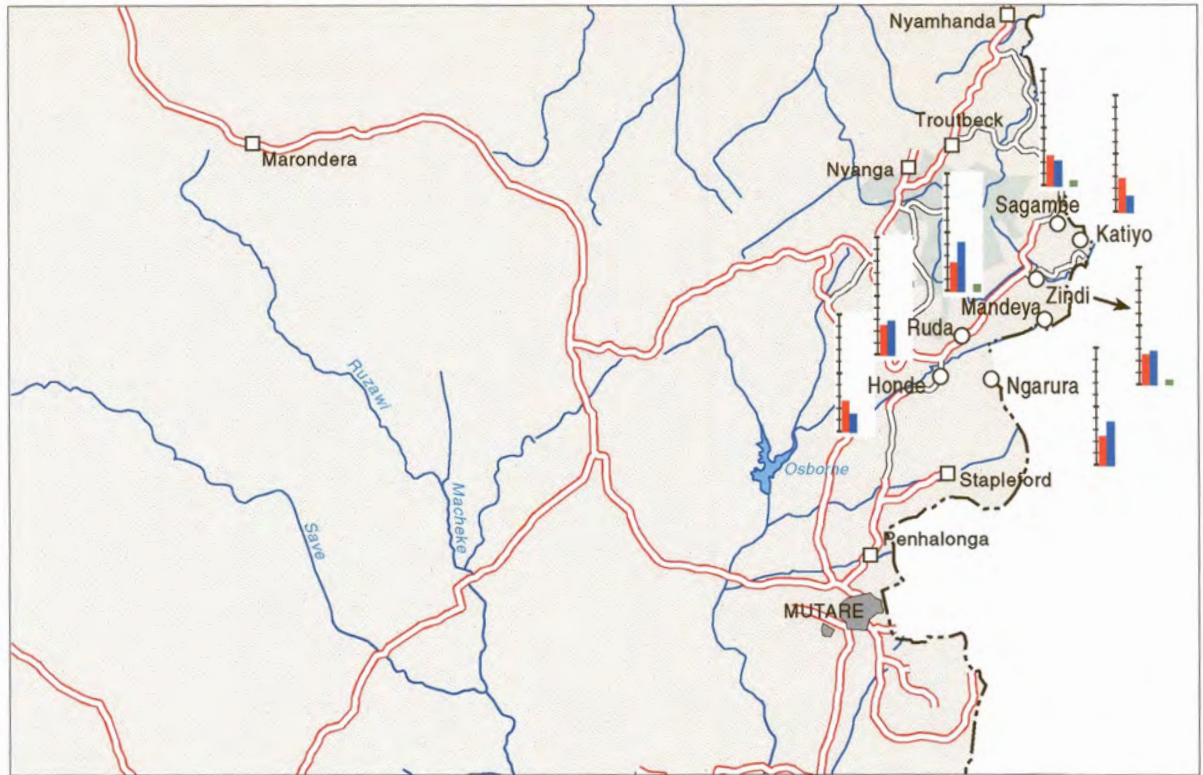
3.6.3 Results

3.6.3.1 Chipinge area

A total of 540 head of cattle, from nine sampling sites, was sampled in the Chipinge area (Table 3.6.1 and Fig.3.6.1). No trypanosomal infections were detected. Anti-trypanosomal antibody levels were present in cattle sampled at each site. However, the average proportion of cattle with anti-trypanosomal antibodies at each sampling site was low (4.8 ± 0.9%). The average percentage positivity of the seropositive samples was 34.0 ± 1.1%. The average PCV of the seropositive animals animals (28.1 ± 0.9%) did not differ significantly from that of the seronegative animals (28.6 ± 0.9%) (P>0.05).

Table 3.6.1: Number of animals sampled, average PCV, proportion of anaemic animals and parasitological and serological prevalence of bovine trypanosomosis at various sampling sites in the Chipinge area.

Sampling site	Number sampled	Average PCV (in %)	Proportion PCV≤24% (in %)	Prevalence (in %)	
				Parasitological	Serological
Mahenye	60	30.6	6.7	0	1.7
Maparadza	60	29.9	5.0	0	5.0
Makoho	60	28.4	11.7	0	8.3
Chinyamukwaka	60	28.9	10.0	0	3.3
Nyamakamba	60	27.3	20.0	0	5.0
Chinyamatika	60	25.5	10.0	0	5.0
Zamuchiya	60	27.5	15.0	0	1.7
Muumbe	60	28.4	20.0	0	3.3
Mwangazi	60	27.9	11.7	0	10.0

Figure 3.6.2: Herd average PCV <UNIVERSITEIT VAN PRETORIA / UNIVERSITY OF PRETORIA / YUNIBESITHI YA PRETORIA> aemic animals, parasitological and serological prevalence of bovine trypanosomosis in the Chipinge area and the Honde Valley in Zimbabwe

LEGEND

Tarred Road		National Land ie. Park, Game & Forest Reserve	
Other Road		International Boundary	
Railway		Settlement	□ Kanzamba
Lake		Sample Site, name	○ Simatele
Pan, Swamp			

Average packed cell volume (%) — (red)
Animals PCV<24%(%) — (blue)
Parasitological prevalence (%) — (black)
Serological prevalence — (green)

100%
50%
0%

171
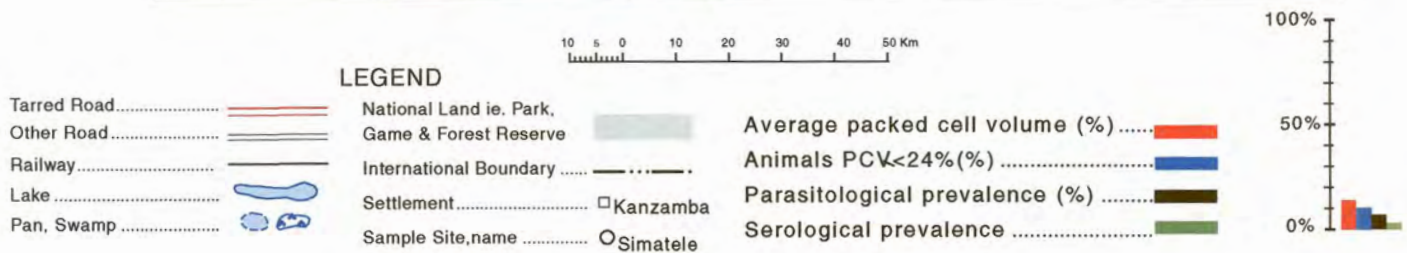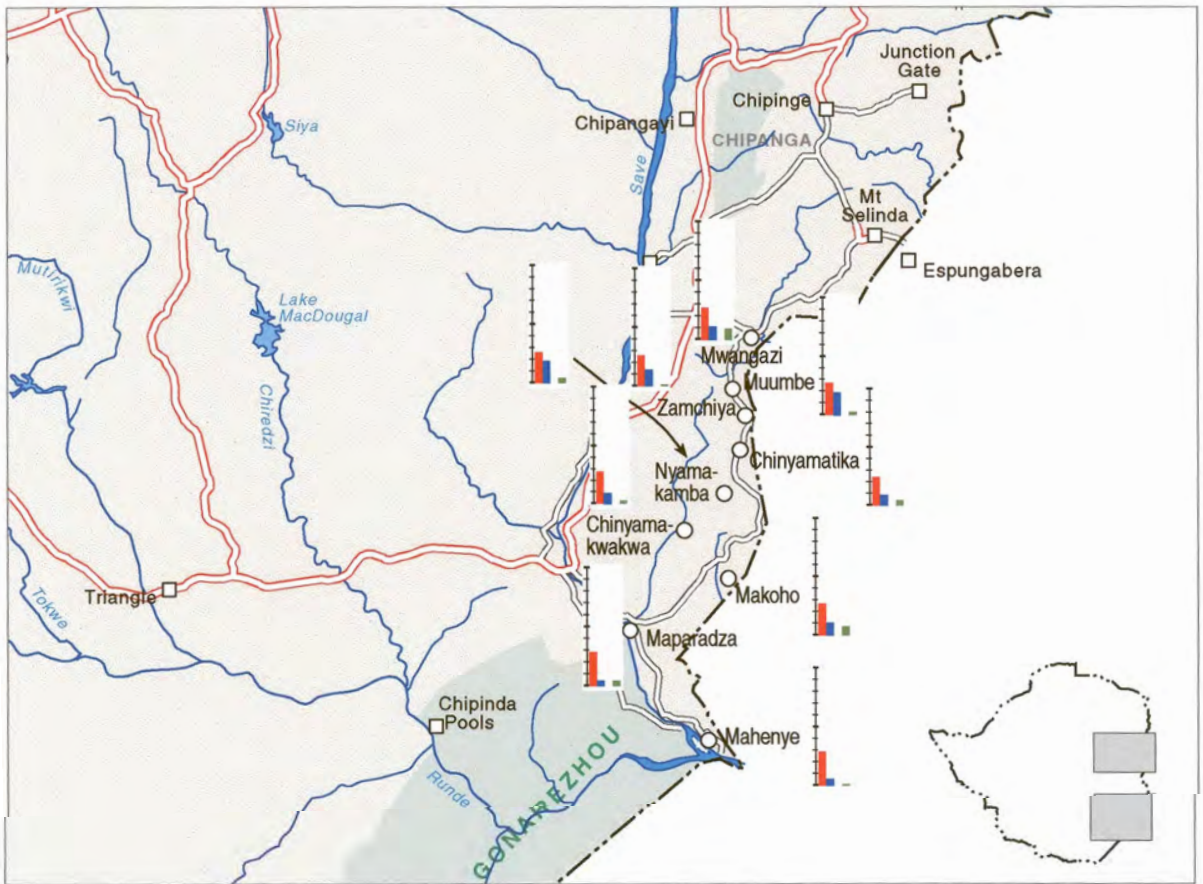
3.6.3.2 Honde Valley

In the Honde Valley 363 head of cattle were sampled at seven sampling sites. No trypanosomal infections were detected. Cattle with anti-trypanosomal antibodies were present at three of the seven sites (Table 3.6.2 and Fig. 3.6.2). The average PCV of the seropositive animals (26.0 ± 1.2%) did not differ significantly from that of the seronegative animals (26.8 ± 0.3%) (P>0.05).

Table 3.6.2: Number of animals sampled, average PCV, proportion of anaemic animals and parasitological and serological prevalence of bovine trypanosomosis at various sampling sites in the Honde Valley, Zimbabwe.

Sampling site	Number sampled	Average PCV (in %)	Proportion PCV ≤ 24% (in %)	Prevalence (in %) Parasitological	Serological
Ngarura	50	25.9	38.3	0	0
Honde	60	27.6	16.7	0	0
Ruda	60	26.1	30.0	0	0
Mandeya	60	25.5	43.3	0	7.1
Zindi	60	27.0	30.0	0	5.0
Katiyo	20	30.0	15.0	0	0
Sagambe	53	27.3	22.6	0	5.6

3.6.3.3 Eastern/northeastern region

A total of 1 594 head of cattle was sampled at 23 sampling sites. Trypanosomal infections (25 *T. congolense* and 13 *T. vivax*) were detected in cattle from four sampling sites most of them located in Mudzi District or adjacent to the target barrier (Table 3.6.3 and Fig. 3.6.3).
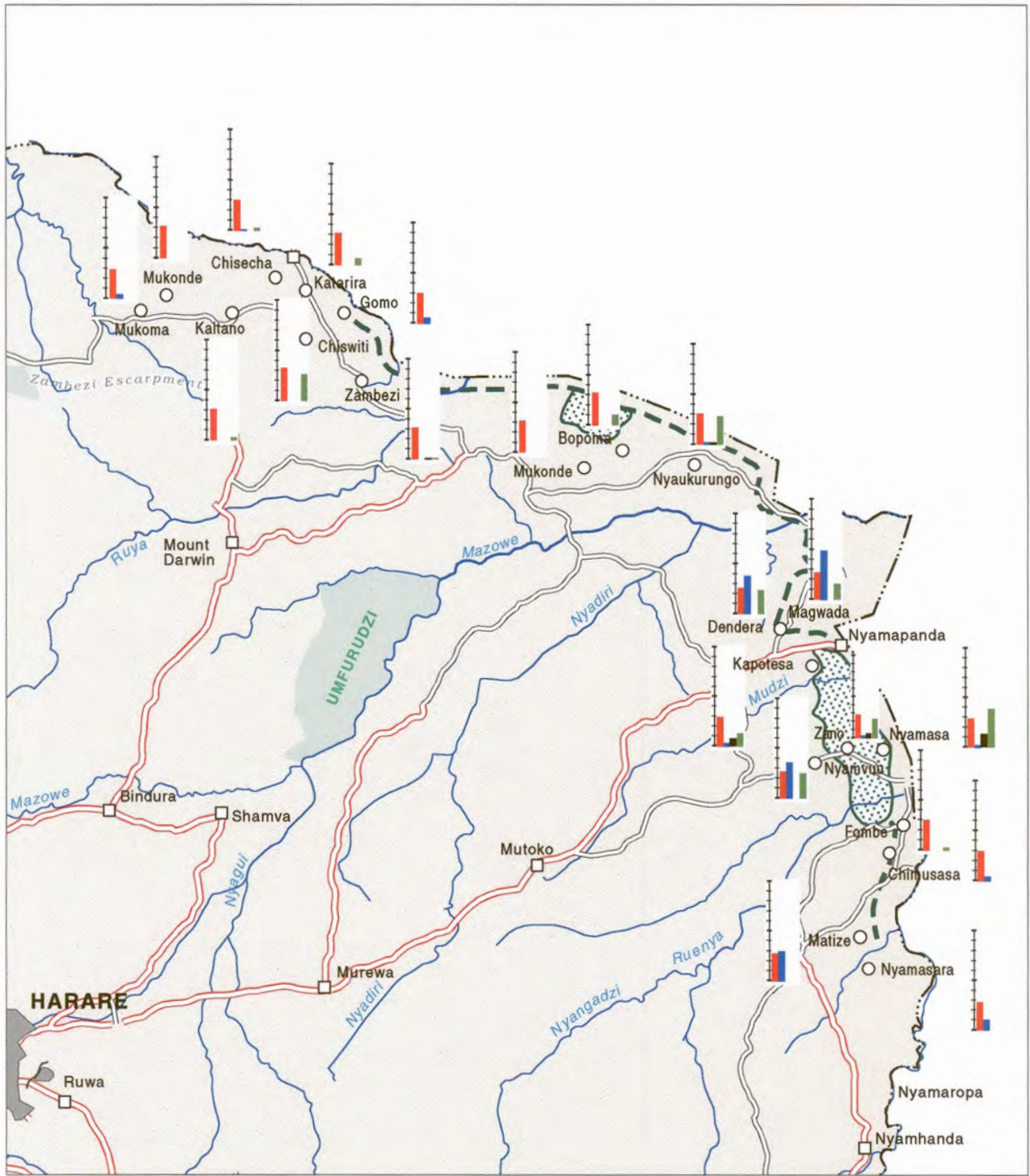
Anti-trypanosmal antibodies were most prevalent in cattle sampled in Mudzi and Guruve Districts. With the exception of cattle sampled at Nyaukurungo and Chiswiti,

anti-trypanosomal antibodies were almost absent in cattle sampled in the remaining parts of the east/northeastern region (Table 3.6.3). The average PCV of all seropositive animals (27.2 ± 0.2%) differed significantly from that of the seronegative animals (29.8 ± 0.2%) (P<0.001).

Table 3.6.3: Number of animals sampled, average PCV, proportion of anaemic animals and parasitological and serological prevalence of bovine trypanosomosis at various sampling sites along the eastern/northeastern region. Zimbabwe.

Sampling site	Number sampled	Average PCV (in %)	Proportion PCV ≤ 24% (in %)	Prevalence (in %)	
				Parasitological	Serological
Nyamasara	60	29.0	11.2	0	0
Matize	60	28.1	30.5	0	0
Chimusasa	60	30.5	5.5	0	0
Fombe	60	31.0	0	0	3.6
Nyamvuu	60	27.7	36.2	0	25.9
Nyamasa	208	28.9	2.4	12.9	38.6
Zano	60	28.2	3.6	5.9	23.4
Kapotesa	112	29.4	1.8	8.3	13.4
Magwada	60	27.5	50.0	0	16.3
Dendera	60	26.8	38.8	0	24.6
Nyaukurongo	64	32.1	3.2	3.1	29.2
Bopoma	60	33.7	0	0	10.9
Mukonde	60	32.7	0	0	0
Zambezi	60	32.7	0	0	0
Gomo	65	29.7	0	0	1.6
Chiswiti	60	33.9	0	0	27.7
Katarira	60	32.5	0	0	7.3
Chisecha	60	31.0	1.7	0	3.6
Kaitano	60	32.3	0	0	3.4
Mukonde	60	32.7	0	0	0
Mukoma	65	29.9	4.6	0	0
Dande	60	31.1	10.0	0	15.7
Mashumbi	60	31.6	1.7	0	27.6

Figure 3.6.3: Herd average PCV, prop... animals, parasitological and serological prevalence of bovine trypanosomosis in the eastern/northeastern region in Zimbabwe.
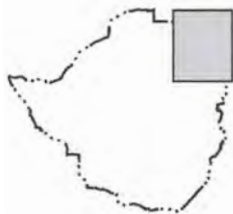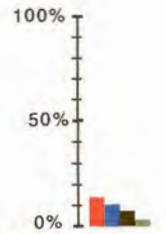
3.6.3.4 Northern region

A total of 762 head of cattle was sampled at 16 sampling sites in the northern region. Trypanosomosis was diagnosed in 93 animals from 11 sampling sites (Tables 3.6.4 and 3.6.5) most of them located within or north of the active target operations (Fig. 3.6.4). Trypanosomal infections were mainly due to *T. congolense* (50.3%) and *T. vivax* (40.8%) (Table 3.6.4).

The average prevalence of anti-trypanosomal antibodies was high in all areas (Table 3.6.5). It was, however, substantially higher in cattle sampled at sites north of the target operations (34.5 ± 7.2%) compared to cattle sampled within (13.5 ± 7.6%) or south (12.4 ± 1.5%) of the target operations.

Table 3.6.4: Number of trypanosomal infections detected and trypanosome species involved at various sampling sites in the northern region, Zimbabwe.

Sampling site	Number sampled	Trypanosome species		
		T. congolense	*T. vivax*	*T. brucei*
Chikova	67	16	1	0
Kadunga	66	1	1	0
Chitindiva	73	10	9	1
Childerly Farm	41	1	0	0
Village 22A	44	1	0	1
Mashongwe	25	1	1	0
Village 32A	76	6	20	0
Rukometjie	32	5	1	3
BH2	46	3	4	0
Makuti	23	2	1	3
Sapi	22	1	0	0

Table 3.6.5: Number of animals sampled. average PCV, proportion of anaemic animals and parasitological and serological prevalence of bovine trypanosomosis at various sampling sites in the northern region, Zimbabwe.

Sampling site	Number sampled	Average PCV (in %)	Proportion PCV ≤ 24% (in %)	Prevalence (in %)	
				Parasitological	Serological
Chibara	60	27.1	47.2	0	11.1
Chengweha	61	26.5	43.8	0	10.7
Chikova	67	24.1	67.5	25.4	28.6
Kadunga	66	26.1	54.4	3.0	15.3
Chitindiva	73	23.3	59.5	27.4	52.7
Nebo Farm	6	29.2	0	0	0
Childerly Farm	41	36.7	6.1	2.4	5.6
Village 22A	44	28.8	46.6	4.5	24.4
Mashongwe	25	29.6	85.4	8.0	13.6
Village 32A	76	24.6	67.5	34.2	49.3
Rukometjie	32	27.8	28.1	28.1	49.3
BH2	46	26.2	21.7	15.2	12.5
Makuti	23	30.7	26.1	26.1	37.1
Sapi	22	31.4	0	4.5	6.2

Figure 3.6.4: Herd average PCV, proportion of anaemic animals, parasitological and serological prevalence of bovine trypanosomosis in the northern region in Zimbabwe.
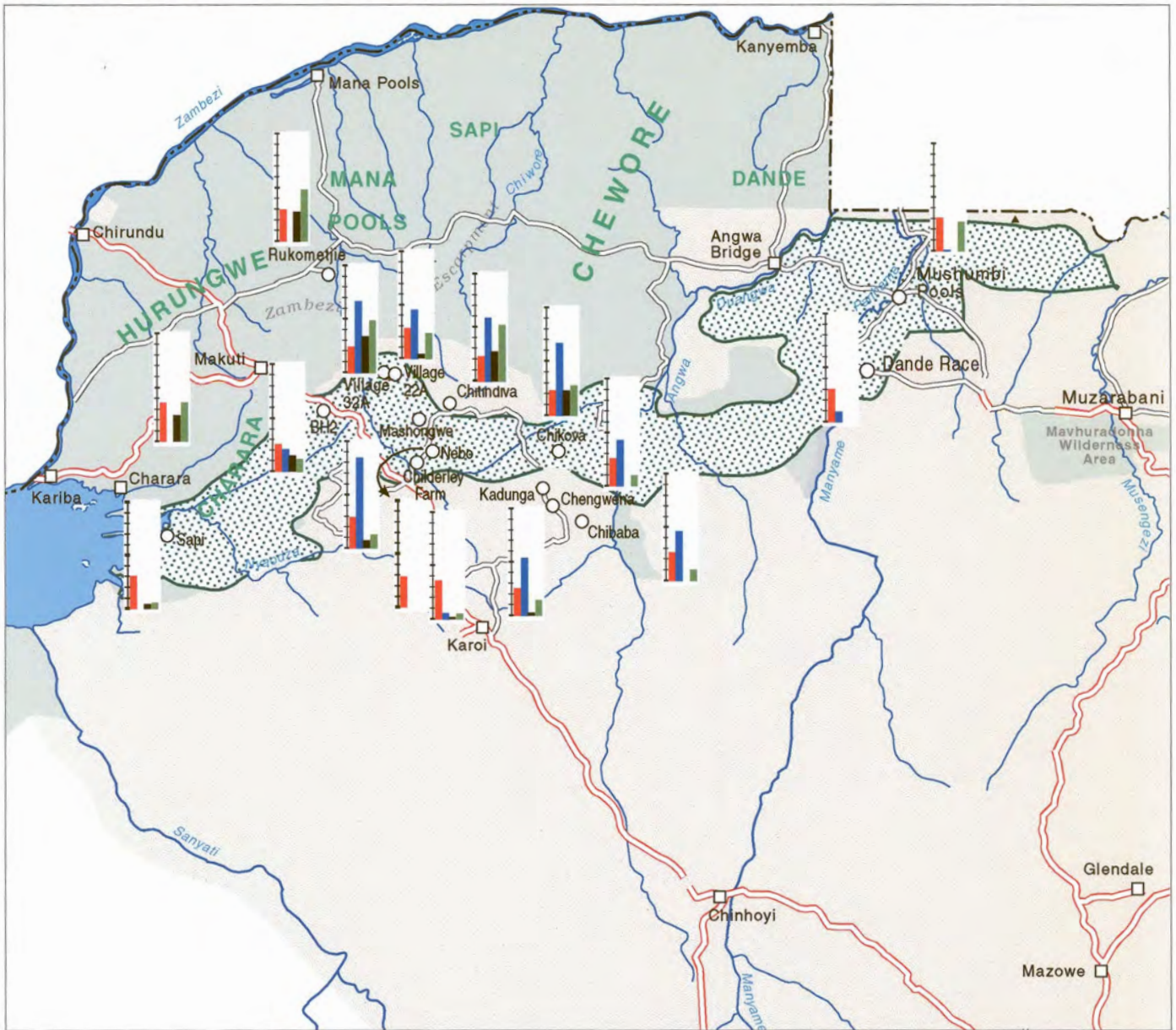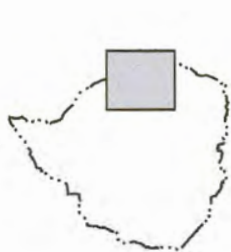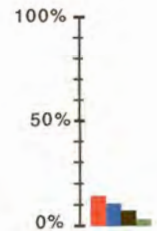

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3.6.3.5 Western region

A total of 1 441 head of cattle was sampled at 25 sampling sites in the western region. Trypanosomal infections (4 *T. congolense*) were detected in cattle sampled at two sampling sites (Table 3.6.6). They were both located south of the tsetse-infested Matusadona National Park (Fig. 3.6.5).

Table 3.6.6: Number of animals sampled, average PCV, proportion of anaemic animals and parasitological and serological prevalence of bovine trypanosomosis at various sampling sites in the western region, Zimbabwe.

| Sampling site	Number sampled	Average PCV (in %)	Proportion $PCV \leq 24\%$ (in %)	Prevalence (in %)	
				Parasitological	Serological
Gandavaroyi	60	29.9	25.0	0	8.3
Tenda	60	26.8	47.2	0	11.7
Nyamatsito	61	28.2	21.8	1.6	5
Nyamasaka	60	28.9	19.5	0	1.7
Guguhwe	26	27.6	74.0	11.5	26.9
Dekete	60	27.4	33.3	0	1.7
Mashama	62	27.5	45.6	0	5.8
Vumba	61	32.2	13.8	0	0
Mola	30	33.4	0	0	0
Zhamba	60	29.3	11.2	0	30.0
Nenyunga	60	28.5	11.2	0	16.7
Nagangala	60	29.7	11.2	0	26.7
Mucheni	60	30.3	11.2	0	11.7
Choto	60	n/a	n/a	n/a	5.0
Gwetsanga	60	32.3	5.5	0	36.7
Sianzali	60	30.8	8.3	0	7.1
Byo Kraal	60	32.5	0	0	1.7
Simatele	60	32.1	2.8	0	8.3
Siyangundu	60	32.3	2.8	0	3.3
Mlibizi	60	31.0	2.8	0	1.7
Kanywambizi	61	30.5	5.5	0	0
Gavu	60	31.5	11.2	0	13.3
Jabula	60	31.5	11.2	0	31.5
Chisuma	60	29.7	8.3	0	0
Sidobe	60	32.1	0	0	10.0

n/a = not available

Figure 3.6.5: Herd average PCV, proportion of anaemic animals, parasitological and serological prevalence of bovine trypanosomosis in the western region in Zimbabwe.
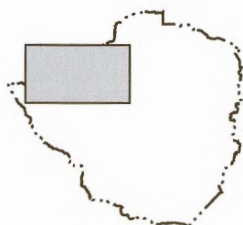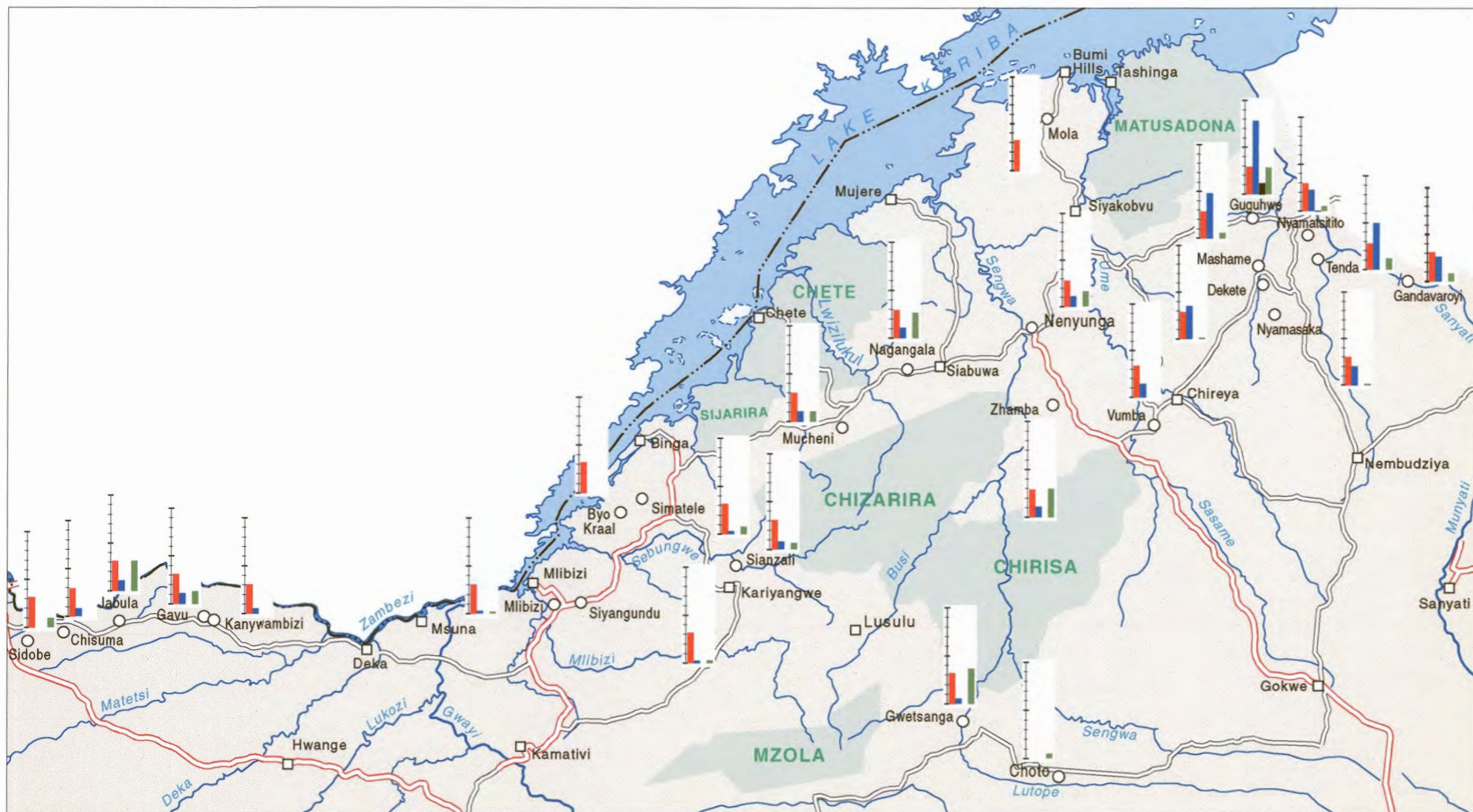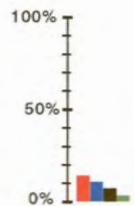
LEGEND

Tarred Road ....
Other Road.......................
Railway ....
Lake ....
Pan, Swamp ....

National Land ie. Park,
Game & Forest Reserve
International Boundary ..
Sample Site, name........... ○Dande
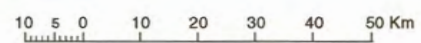
Average packed cell volume (%) ....
Animals PCV≤24%(%) ....
Parasitological prevalence (%)..........
Serological prevalence.......................

10 5 0   10   20   30   40   50 Km

100%

50%

0%

The prevalence of cattle with anti-trypanosomal antibodies was relatively high at samplings sites located along the Sanyati River, in the vicinity of the Chizarira National Park and the Chirisa Safari Area and along the border with Zambia in the vicinity of Victoria Falls.

3.6.4 Discussion and conclusions

3.6.4.1 Distribution and prevalence of bovine trypanosomosis

(i) Chipinge area

Whereas no trypanosomal infections were diagnosed using parasitological diagnostic methods, anti-trypanosomal antibodies were detected in some of the animals at all sampling sites. However, the average prevalence of cattle with antibodies was low. Moreover, since the anti-trypanosomal antibody detection ELISA is not 100% specific and sensitive, a proportion of these positive reactions are likely to be false positives. This proportion of false positives can even increase with decreasing prevalence of infection (Thrusfield. 1986). Such false positive reactions may partly explain the very low prevalence of cattle with anti-trypanosomal antibodies at most of the sampling sites. However, the prevalence of anti-trypanosomal antibodies in cattle sampled at Makoho and Mwangazi does suggest a degree of tsetse challenge. Since anti-trypanosomal antibodies persist, these antibody levels could be the due to a current trypanosomal infection that was not detected or an infection that was cured within a period of a maximum of 13 months previous to sampling (section 3.2.3). Because of the low sensitivity of parasitological methods for diagnosing trypanosomosis, a substantial proportion of the trypanosomal infections were not diagnosed (Paris *et al.*, 1982). Hence. some animals with anti-trypanosomal antibodies may, in fact. have be infected with trypanosomes. If this was the case in this survey. the average PCV of seropositive animals could be expected to be lower than that of seronegative animals. This was not the case. The results. therefore. suggest that trypanosomosis challenge in the Chipinge area is low and probably irregular. This was confirmed by the absence of tsetse catches during a 12-month intensive tsetse surveillance exercise in the Chipinge area (Chihiya. personal communication. 2000) Nevertheless. more focused surveillance is required to

elucidate the origins of the challenge. Moreover, surveillance units need to be established to monitor the possible spread of tsetse in the Gonarezou National Park.

(ii) Honde Valley

In the Honde Valley, only three sampling sites had cattle with anti-trypanosomal antibodies. At each of these sites, the prevalence of cattle with anti-trypanosomal antibodies was low. Moreover, the average PCV of seropositive animals did not indicate the presence of active trypanosomal infections. As in the Chipinge area, the Honde Valley survey results suggest that a low degree of tsetse challenge was present. This is not surprising because of the absence of tsetse control measures and the possible presence of tsetse species such as *G. austeni* that would be difficult to detect. Regular monitoring of the situation is required.

(iii) East/northeastern region

Along the east/northeastern border with Mozambique, tsetse challenge was highest in Mudzi, in the southern part of Rushinga and in Gururve Districts. Despite the presence of a 10 km-wide target barrier in Mudzi District, several trypanosomal infections were diagnosed and high proportions of animals had anti-trypanosomal antibodies. This challenge is attributed to the high invasion pressure of tsetse (*G. m. morsitans* and *G. pallidipes*) along this part of the Zimbabwe/Mozambique border (Section 5.6.3.2). Whereas entomological and disease prevalence data indicate that targets are able to cope with this invasion pressure, cattle are likely to be challenged when grazing close to the tsetse invasion front.

In areas south and north of the Mudzi District, the parasitological prevalence of trypanosomosis and the prevalence of anti-trypanosomal antibodies was low. These results suggest that the areas have been cleared effectively of tsetse and that the control measures left in place cope effectively with the invasion pressure from neighbouring Mozambique. Exceptions were the relatively high prevalence of anti-trypanosomal antibodies in cattle sampled at Magwada and Dendera (Rushinga District), at Katarira (Centenary District), at Chiswiti (Centenary District) and Dande and Mashumbi (Gururve District) and the presence of a trypanosomal infection and anti-trypanosomal antibodies in cattle sampled at Nyaukurongo (Mount Darwin

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District). The anti-trypanosomal antibody levels detected in cattle sampled at Katarira and Chiswiti could be explained by the presence of a residual tsetse focus. In 1996, tsetse flies (*G. m. morsitans*) were captured southeast of Mukumbura (Davison, 1996). However, a more detailed assessment of the situation is required. The origin of the trypanosomal infection detected in Nyaukurongo is not clear. It could be attributed to the movement of cattle into tsetse-infested habitat in Mozambique or the reinvasion by tsetse. Again, more detailed investigations are required. Since Mashumbi is located close to the tsetse invasion front (Fig. 3.6.3), the high prevalence of cattle with antibodies (27.7%) is not surprising. Challenge still occurs at Dande but at a much lower rate compared to Mashumbi. According to these results, the effectiveness of the target barrier in Rushinga District needs to be reviewed.

(iv) Northern region

Not surprisingly, the prevalence of trypanosomal infections and anti-trypanosomal antibodies was high in animals sampled north of the target-protected area. *Trypanosoma congolense* and *T. vivax* were the dominant trypanosome species diagnosed. In southern Africa, *T. congolense* is the most prevalent trypanosome species in cattle (Section 3.4). The high proportion of *T. vivax* infections detected in the northern region was, therefore, unusual. Since *T. vivax* has a short developmental cycle in the tsetse fly (Davies, 1977), a high proportion of *T. vivax* is expected in younger age-groups of tsetse. Hence, *T. vivax* infections are likely to dominate in areas where the proportion of young tsetse flies is high because of high mortality rates. Such high mortality rates certainly occur within or along a target barrier.

South of the target barrier, only one trypanosomal infection was detected in an animal sampled at Kadunga. However, the proportion of cattle with anti-trypanosomal antibodies suggests challenge at all other sampling sites located south of the target barrier. These findings are not surprising since cattle immediately south of the target barrier can move into the barrier zone and become infected. Furthermore, the presence of tsetse south of the barrier cannot be excluded.

(v) Western region

In the Western region, trypanosomal infections were detected in cattle sampled along the Sanyati River and south of the tsetse-infested Matusadona National Park. The infections south of the National Park indicate that tsetse were present south of the target barrier surrounding the National Park. Hence, before commencing with the clearing of tsetse from the Matusadona National Park, detailed information on the spread of the fly outside the National Park's boundaries should be obtained. The trypanosomal infections detected in cattle sampled along the Sanyati River could be due to movement of tsetse from the Matusadona National Park. However, tsetse (*G. pallidipes*) have been captured in large numbers in this area suggesting that residual foci of tsetse may still be present (TTCB, 1991).

According to the survey results, bovine trypanosomosis was absent from most areas sampled along the southern shoreline of Lake Kariba. However, trypanosomal antibodies were prevalent in cattle sampled at sites surrounding the Chizarira National Park and the Chirisa Safari Area. This suggests that, despite the efforts made to clear the area from tsetse (Lovemore, 1990), challenge still occurs. Intensive tsetse surveys are required to elucidate the tsetse situation.

A high proportion of cattle with anti-trypanosomal antibodies was detected along the Zambezi River in the western part of the country. The most likely explanation for this occurrence is the southerly advance of the *G. m. centralis* fly belt in Zambia or movement to the west of tsetse (*G. m. morsitans* or *G. pallidipes*) from the Lusitu area (Southern Province, Zambia). Indeed, bovine trypanosomosis was detected in cattle sampled on the other side of the Zambezi River immediately north of Jabula (Lovemore, 1989; RTTCP, 1991). A high level of vigilance is urgently required.

3.6.4.2 Monitoring of the effectiveness of tsetse control interventions

The effectiveness of tsetse control operations is assessed by establishing their impact on the density of the tsetse population. Once tsetse have been cleared and the threat of reinvasion remains, continuous monitoring is required to confirm the tsetse-free status. This was in progress in large areas of Zimbabwe. Monitoring may either be direct or indirect. Direct monitoring involves determining the presence or absence of tsetse. This approach has the obvious advantage that, if flies are captured,

conclusions are unequivocal. Moreover, this type of monitoring can be conducted in all areas since it does not rely on the presence of cattle. However, interpretation of results is difficult when no tsetse are caught. The absence of tsetse catches in traps or along fly-round transects does not necessarily mean the total absence of the fly (Hargrove, 1980a). Therefore, information on the abundance of tsetse is supplemented usually by indirect indicators of the presence of tsetse. Useful indirect indicators are data on the parasitological prevalence or incidence of bovine trypanosomosis in sentinel herds or locally owned cattle. Information on the prevalence or incidence of nagana is particularly helpful when trypanosomal infections are detected in areas where, according to the outcome of tsetse samplings, no flies are present. However, interpreting results is difficult when no trypanosomal infections are detected. Indeed, the absence of detectable trypanosomal infections does not necessarily mean the absence of the disease. The currently available methods for the parasitological diagnosis of trypanosomosis have variable but generally low sensitivity (Paris *et al.*, 1982) and many infections are not detected. Although this may not be such a problem in areas where nagana is highly prevalent, the low sensitivity has important consequences in areas where the disease is present at low prevalence. The latter areas are usually those where tsetse, because of their low population density, cannot be captured with the conventional sampling methods. The low sensitivity of the tsetse sampling methods and methods for the parasitological diagnosis of trypanosomosis can be compensated for by surveillance. However, surveillance is time consuming and labour intensive. Therefore, it is frequently beyond the financial means of a tsetse control department. Moreover, whereas surveillance may be used to declare an area vector-free it is practically impossible to continue such intensive surveillance to monitor the vector-free status of the whole tsetse-cleared area in, for example, Zimbabwe. Clearly there is a need for a simple and relatively inexpensive method to assess quickly and accurately the effectiveness of control measures. The anti-trypanosomal antibody detection ELISA may meet this requirement. The sampling procedure has been simplified. Whole blood samples can be collected on filter papers, which makes the technique user-friendly and less expensive (Hopkins *et al.*, 1998). It also dispenses with the need for a cold chain. More importantly, the test is robust and has high sensitivity and specificity. The test detects antibodies against current and past infections. Although this is often considered as a disadvantage, it may be an

advantage when the antibody-ELISA is used to monitor the tsetse-free status of an area. Indeed, the anti-trypanosomal antibody levels in a herd will give an indication of the presence or absence of challenge during the previous 13 months (Section 3.2.3). Like most diagnostic methods with specificity of less than 100%, false positive reactions may make interpretation of results difficult. This is especially the case when challenge is low or absent (Thrusfield, 1986). Indeed, even in the absence of challenge, false positive reactions are likely to occur. Ideally, when prevalence is very low, a more sensitive and specific test is required. Such a test is not available for routine use. In practice, however, repeated testing of the same specimen can solve the problem of false positive reactions.

From the results of the survey conducted in Zimbabwe, the antibody-ELISA is a useful monitoring tool in areas where;

1) tsetse have been absent for many years but where the threat of tsetse reinvasion is present (Chipinge area, Honde Valley and western region);

2) the effectiveness of barriers to reinvasion of tsetse needs to be assessed (east/northeast and northern regions);

3) residual tsetse foci may be present (western region)

Although monitoring should not rely solely on the antibody-ELISA, it is certainly a useful additional tool in situations where a high degree of sensitivity and specificity is required. The test can only be applied to those areas where cattle are present but its results do not indicate where and when challenge occurred. However, in some cases it may be useful to introduce seronegative sentinel cattle. By monitoring the serological status of the sentinel animals over time and accurately establishing their movement patterns, useful information can be obtained on the time and place of challenge.

Despite routine tsetse surveillance in Zimbabwe, the results obtained from the survey described in this paper have improved greatly the knowledge of the bovine trypanosomosis situation in Zimbabwe. Similar follow-up surveys could be conducted at, for example, six-monthly intervals or when required.