A CROSS-SECTIONAL EPIDEMIOLOGICAL SURVEY OF BOVINE TRYPANOSOMOSIS AND ITS VECTORS IN THE SAVELUGU AND WEST MAMPRUSI DISTRICTS OF NORTHERN GHANA

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Abstract

The epidemiology of bovine trypanosomosis was investigated in two districts (Savelugu and West Mamprusi) of Northern Ghana with different land use and environmental characteristics. The land use intensity and environmental change was suspected to be higher in the Savelugu District. A cross-sectional entomological survey conducted along the White Volta river and its tributaries confirmed the presence of only *Glossina palpalis gambiensis* and *G. tachinoides*. The challenge index as measured by the product of tsetse density and tsetse infection rate was much higher in the West Mamprusi (19.6) than in the Savelugu district (4.7). A total of 1,013 cattle (508 in Savelugu and 505 in West Mamprusi) were bled from a random selection of 16 villages in the Savelugu District and 13 villages in the West Mamprusi District. Blood samples were examined for trypanosomes by the buffy coat technique (BCT). Blood samples that were positive in the BCT or negative in the BCT but with Packed Cell Volume (PCV) values below 21 were further tested with a polymerase chain reaction for trypanosomal DNA. Plasma samples of all cattle were serologically tested with an indirect ELISA for trypanosomal antibodies. The parasitological and serological prevalence of bovine trypanosomoses was significantly higher in West Mamprusi (16 and 53%, respectively) than in Savelugu District (8 and 24%, respectively). An evaluation of animal health at the village herd level, using PCV as an index of anaemia, provided various epidemiological scenarios prevalent in the entire study area.

Keywords : cattle-Protozoa, tsetse fly, trypanosomosis, survey, epidemiology, Ghana

1. Introduction

The direct and indirect impacts of African Animal Trypanosomosis on agriculture constitute a major constraint to the socio-economic development of tsetse fly (*Glossina*) infested areas of Africa (Swallow, 1998). Various options are available for the control of animal trypanosomosis ; they include the suppression and/or eradication of tsetse populations (Jordan, 1986), the use of trypanotolerant breeds of livestock (D'Ieteren et al., 1998), chemotherapy (Peregrine, 1994) or combinations of these (Holmes, 1997).

Studies conducted in West Africa amply demonstrate that decision making on the control of tsetse-transmitted trypanosomosis, at the regional level, should be founded on an objective assessment of the impact of the disease on production systems (Hendrickx et al., 1999). At the local level, it is important that due cognisance is given to the dynamic nature of

trypanosomosis, and in particular to the evolution of the disease with human population growth and agricultural expansion (Bourn et al., 2001). Although the *morsitans* group of tsetse flies (Subgenus *Glossina* s. str.) is expected to decline appreciably with demographic pressure, the more tenacious *palpalis* (Nemorhina) group of tsetse flies could continue to persist in isolated habitats (Reid et al., 2000). A study conducted on an agro-pastoral zone of Burkina Faso, showed that changes in the distribution of *Glossina palpalis gambiensis* Vanderplank and *Glossina tachinoides* Westwood, over a 15 year period, were partially due to the proximity of cropping to river banks (de La Rocque et al., 2001); the said study did not, however, state the impact of such changes in fly distribution on the epidemiology of trypanosomosis.

This present survey sought to obtain and compare prevalence estimates of bovine trypanosomosis, transmitted by tsetse flies of the *palpalis* group, in two geographically contiguous areas (Savelugu and West Mamprusi Districts of Northern Ghana) with different land use patterns and to elucidate prevailing epidemiological scenarios in the two districts.

2. Materials and methods

2.1. Study area

The two districts are located in the northern part of Ghana at the limits of the Guinea and Sudano-Guinean savanna zones. The riparian vegetation of the main river, which is locally referred to in Ghana as the "White Volta" and its tributaries are infested with *G. palpalis gambiensis* and *G. tachinoides* (Draeger, 1983). In the Savelugu District, which has a relatively high human population (60 people /km^2), some segments of riparian vegetation have been obliterated by high human activity causing the disappearance of potential habitat for tsetse flies. The West Mamprusi District on the other hand has a low human population density of 22 people /km^2 (Nippah, 1997: personal communication). Encroachment on riparian vegetation in this district is minimal and hence tsetse habitat is relatively undisturbed. Contact between tsetse flies and cattle occurs when cattle are herded along the White Volta.

2.2. Tsetse survey

A cross-sectional tsetse fly survey was conducted in the dry season (February-June 2001) along the White Volta and its tributaries in the Savelugu and West Mamprusi Districts. Biconical traps (Challier and Laveissière, 1973) were deployed at an interval of about 100-200 meters along riparian vegetation. The coordinates of each trap position were recorded with a Global Positioning System (GPS). Trapping sites corresponded to those visited by

livestock and man for water. A total of 13 sites (5 sites in Savelugu and 8 sites in West Mamprusi) were surveyed. Ten traps were deployed per site making it a total of 130 traps for the entire study area. Tsetse flies were collected from each trap twice a day for a period of 48 hours and sorted according to species.

2.3. Detection of trypanosome infection in tsetse flies

Tsetse flies caught were dissected in normal saline and the hypopharynx, midgut and salivary glands examined under a compound microscope for trypanosome infection. Infected *Glossina* parts were preserved in 70% alcohol in Eppendorf tubes until they reached the laboratory (CIRDES, Burkina Faso) and were treated with Chelex® as previously described by Desquesnes and Dávila (2002). They were further tested for trypanosomal deoxyribonucleic acid (DNA) using the Polymerase Chain Reaction (PCR) as described by Masiga et al. (1992), using primers specific for *Trypanosoma vivax, T. congolense* (savannah type) and *T. brucei*. Tsetse flies that were dessicated were rejected.

2.4. Survey of bovine trypanosomosis

2.4.1 Sampling

A multistage sampling method was used. The two districts were considered as geographical entities separated by administrative boundaries and were purposively selected because of differences in environmental characteristics. About 30% of villages were randomly selected in each zone (16 villages in Savelugu and 13 villages in West Mamprusi). Assuming an estimated trypanosomosis prevalence of 50% and with a desired accuracy of 5% at the 95% confidence level, an estimated sample size of 384 cattle was obtained for each zone (Cannon and Roe, 1982). To improve on the reliability of the sample size, more than the required number of samples were taken (508 cattle and 505 cattle in Savelugu and West Mamprusi Districts respectively). In each zone, the number of animals selected in each village was based on proportional weighting. At the village level herds were selected by simple random sampling. The number of cattle sampled from a particular herd in a given village depended on proportional weighting; but a minimum of 20 cattle per herd was fixed. Where a chosen herd did not have the required number of cattle (as calculated by proportional weighting), a second herd was selected by random sampling, and the remaining number of cattle made up by simple random sampling. Geographical co-ordinates of selected villages were also recorded. The sex, age and breed of animals were noted. A questionnaire was

applied to determine the kind of herd management as well as treatment regimes of trypanocidal drugs practiced.

2.4.2 Parasitological diagnosis

Blood was taken into heparinised haematocrit tubes from the ear vein and examined for trypanosomes using the buffy coat technique (BCT) (Murray et al., 1977). The Packed Cell Volume (PCV) was read for each sample. The buffy coat of samples that were positive for pathogenic trypanosomes, and negative samples with PCV values below 21%, were dotted onto filter paper (Whatman Number 1) as described by Omanwar et al. (1999). Trypanosomal DNA was extracted in Chelex® 100 (Solano et al., 1999) and tested as described for flies organs, with primers specific for *T. vivax, T. congolense* (savannah type) and *T. brucei* . A sample was considered positive if it was positive in the BCT and/or the PCR.

*2.4.3 Antibody detection by an enzyme-linked immunosorbent assay (ELISA***)**

The plasma component of each bovine blood sample, after centrifugation, was dotted on filter paper, dried and preserved at ambient temperature (Hopkins et al., 1998). The plasma-impregnated portions of the filter paper were cut out and the plasma eluted with 1.5 ml of phosphate buffered saline (PBS) containing 0.1% Tween 20 for 2 hours on a rotative shaker. Using antigens of *T. vivax* (Zaria 81/Y486/699) (Guidot and Roelants, 1982), *T. congolense* (IL1180)(Peregrine et al., 1991) and *T. brucei* (Cross, 1977) three indirect antibody ELISAs were performed on the samples (Desquesnes et al., 2001); evaluated under controlled conditions these 3 tests exhibited sensitivities >90% in experimentally infected sheep and specifities >99% in non-infected animals (Desquesnes et al., 2001). Four negative control plasma were obtained from 4 non-infected cattle born in the tsetse-free zone of Dori in Burkina Faso. Positive control plasma were obtained from 3 cattle experimentally infected with *T. vivax* (Zaria 81/Y486/699) (Guidot and Roelants, 1982), *T. congolense* (IL1180)(Peregrine et al., 1991) or *T. brucei* (Farakoba/80/CRTA/1) (Roelants et al., 1985), respectively. The plasma were dotted on filter paper and kept at 4°C to be used as controls after similar elution as test samples. For each type of antibody ELISA (ELISA-*T. vivax*, ELISA-*T. brucei* and ELISA-*T. congolense*), results were expressed in Percentage of Positivity (PP) as previously described by Desquesnes (1997). Samples giving a PP which exceeded the mean PP of negative samples plus 3 standard deviations were considered

positive (Desquesnes et al., 2001). A sample was considered positive if it was positive for one or more of the 3 types of ELISA.

3. Results

3.1 *Tsetse fly species distribution, population density and infection rate*

There was a downward gradient in the apparent density of tsetse per site from the northern to the southern limits of the survey area (Fig. 1). Throughout the range of the tsetse survey area, the species found were *G. palpalis gambiensis* and *G. tachinoides*. The apparent density varied widely between sites. In the case of *G. palpalis gambiensis*, the number of tsetse flies caught per trap per day at a given site varied between 0 and 18 with a mean of 2. No *G. palpalis gambiensis* were found at the northern limits of the survey area. In the case of *G. tachinoides* the catch per site varied between 2 and 257 with a mean of 40 flies per site per day; this species of tsetse fly was found throughout the range of area surveyed. The challenge index (apparent density x tsetse infection rate) was appreciably higher in the West Mamprusi District (19.6) than in the Savelugu District (4.7) (Table 1). The trypanosome species infecting flies were *T. vivax, T. congolense* (savannah type) and *T. brucei* (Table 1). In view of the small number of flies that were infected, the relative occurrence of the trypanosome species in tsetse flies can only be qualitatively appreciated.

3.2 Prevalence of bovine trypanosomosis

Using the buffy coat technique (BCT), the mean prevalence of positive samples in the Savelugu District was 8%. The PCR did not detect any samples that were negative in the BCT with PCV values of less than 21% but confirmed over 98% of positive samples detected by the BCT. Among the various cattle breeds the prevalence was 6% (n=325) in the West African shorthorn, 11% (n= 162) in the Sanga and 14% (n= 21) in the Zebu. Using the results of the BCT and PCR in the West Mamprusi District, the prevalence was 16%. The PCR besides agreeing over 95% with the BCT results had detected 4 positive cases out of 41, which could not be detected by the BCT and had PCV values of less than 21%. In the various breeds the prevalence was 19% (n=383) in the West African Shorthorn, 9% (n=82) in the Sanga and 8% (n=40) in the Zebu. In both districts *T. vivax* was the most prevalent trypanosome infection, followed by *T. congolense* (savannah type) and then *T. brucei*, as shown in Table 2. There was a statistically significant difference between the medians of parasitological prevalence of the two districts (U=57.5, P=0.05 Mann-Whitney U-test).

The mean seroprevalence in Savelugu District and West Mamprusi District were 24% and 53%, respectively. There was a statistically significant difference between the medians of serological prevalence in the Savelugu and West Mamprusi Districts (U= 24, P=0.05 Mann-Whitney U-test).

Generally, the serological prevalence of bovine trypanosomoses was higher than the parasitological prevalence. This information is depicted in Fig. 2. Trypanosomal antibodies were detected in cattle less than one year old. Across the age spectrum of cattle in the West Mamprusi District, antibody prevalence was higher in the older age groups but this was less evident in the Savelugu District (Fig. 3). The mean and median age of cattle examined were both 4 years and the range was from 3 months to 20 years.

3.3 Analysis of Packed Cell Volume (PCV)

The mean (± SD) PCV for the West African Shorthorn (WASH), the Sanga and the Zebu were 28.9% ± 0.2 (n=707), 31.4% ± 0.35 (n=244) and 33.3 %± 0.67(n=61), respectively. There was a highly significant statistical difference between the mean PCV of the West African Shorthorn breed and that of the Zebu (P=0.01, Z-test); there was also a highly statistically significant difference between the mean PCV of the West African Shorthorn and the mean PCV of the Sanga (P=0.01, Z-test). A comparison of the mean PCV of the Sanga to the mean PCV of the Zebu showed no significant difference (P=0.01, Z-test).

3.4 Prevalence of trypanosomosis and the occurrence of anaemia

The mean PCV for all cattle in the study area was 29.7 % (± 5.7). A threshold of 21 was chosen below which animals were considered anaemic, because there was a statistically significant difference between PCV values of less than 21% and the population mean. A scatter plot of parasitological prevalence and the prevalence of anaemia at the village level is shown in Figure 4. A threshold of 5% was set for both parasitological prevalence and the prevalence of anaemia above or below which parasitaemia or anaemia were considered as high or low, respectively (shown by vertical and horizontal dotted lines on the graph). Using these thresholds four categories of village herds were identified: those with a low prevalence of trypanosomosis and a low prevalence of anaemia (n=3), those with a high prevalence of trypanosomosis and a low prevalence of anaemia (n=17) ; those with a high prevalence of trypanosomosis and a high prevalence of anaemia (n=8) and finally, those with a low

prevalence of trypanosomosis and a high prevalence of anaemia (n=1). This information is spatially represented in Fig. 5.

4 Discussion

The trypanosome infection rate in tsetse flies was approximately the same for both districts but the tsetse density per site in the West Mamprusi District was almost 4 fold higher than that of the Savelugu District, hence the trypanosome challenge in the former was much higher. Tsetse blood meal analysis would have provided the true trypanosome challenge that cattle in the areas were exposed to. However, blood meal analysis was considered impractical because of the low tsetse fly counts at several sites and the fact that more than 90% of tsetse flies caught were hungry flies. Rogers (1985) observed that while tsetse infection rate tends to be the same in a given area, there can be great variability in tsetse apparent density. The main reason for the differences in tsetse density observed in the two districts is the fragmentation of tsetse habitat along riparian vegetation as described by de La Rocque et al. (2001).

Although variations in humidity and temperature between February and June could have influenced tsetse fly population density from north to south, this was not a major consideration because only 10 traps were set in the month of June at one site at the northern limit of the survey area. The said site has from previous surveys been known to have a high tsetse population density (Draeger, 1983) as was confirmed by the present survey. The other 120 traps were set between the months of February and April during which time temperature and humidity differences between the months were minimal (Amingo, 2001: personal communication). No traps were deployed in the month of May due to accessibility problems.

In both districts, most cattle are herded along the river banks of the White Volta during the dry season, where they come into contact with tsetse flies. In the rainy season, cattle graze and water close to the village and hence the risk of trypanosomosis is minimised. Since cattle management is the same in both districts, the higher serological and parasitological prevalence of bovine trypanosomosis observed in the West Mamprusi district as compared to the Savelugu District was attributed to the higher trypanosome challenge in the former. The parasitological prevalence was probably underestimated because the PCR was used only on samples that were positive with the BCT or samples that were negative with the BCT and had PCV values of less than 21%. Where the two tests are used in parallel the PCR increases the number of cases detected (Solano et al., 1999).

The pattern of serological prevalence in the different age groups, observed in the West Mamprusi District is consistent with the findings of Desquesnes et al. (1999). Older animals had more chances to be exposed to tsetse bites than younger ones. The higher serological prevalence as compared to parasitological prevalence observed in both districts at the village level, suggests that more animals were exposed to trypanosome infection but had undergone self-cure or had parasitaemias that were too low to be detected by both the PCR and the buffy coat technique. The occurrence of *T. congolense* (savannah type) as revealed by the PCR is significant from the veterinary point of view as this type is known to be more virulent than other *T. congolense* types (Bengaly et al., 2002).

The significant difference in the PCV values observed for the West African Shorthorn, the Sanga and the Zebu makes it imperative to consider these breeds separately when evaluating animal health, especially in small herds. The use of trypanocidal drugs and the susceptibility of trypanosome populations to these drugs could have confounded not only the conclusions drawn about the PCV values observed in the different cattle breeds but also about the prevalence of trypanosomosis in the two districts. However, treatment history provided by the owners of all cattle examined indicated that they were last treated with trypanocides (diminazene derivatives) on average 6 months before the survey. Although resistance to trypanocidal drugs has not yet been reported in Ghana, reports of drug resistance in other parts of West Africa (Clausen et al., 1992; Peregrine, 1994) suggest that this phenomenon should be taken into account when interpreting parasitological prevalence figures. Anaemia is an index of animal health in bovine trypanosomosis. In epidemiological surveys, the focus should be on anaemia rather than parasitaemia alone because parasitaemia may just be an indicator of carrier status, especially in trypanotolerant cattle (Hendrickx et al., 2001). In this study it was observed that cattle in 17 out of 29 villages had a high parasitological prevalence but generally had normal haematocrit values. This may be explained by the high proportion of trypanotolerant cattle in the study area. There were also 8 villages in which there was a high prevalence of trypanosomosis and a high prevalence of anaemia ; in this situation, trypanosomosis was considered a major problem. Out of the said 8 villages, 6 villages were found in the West Mamprusi District, which is not surprising given the high challenge index in this region. Despite the low prevalence of trypanosomosis in the Savelugu District, cattle in some villages were adversely affected by the disease. Overall, there was only one village in which cattle had a high prevalence of anaemia, despite the low prevalence of trypanosomosis; anaemia in this situation could be attributed to some other causes such as fasciolosis or haemonchosis as reported elsewhere in the West-African sub-region (Ndao et al., 1995;

Zinsstag et al., 1998). Finally, there were 3 villages where the prevalence of trypanosomosis and the prevalence of anaemia were both low; it is assumed that in this category, trypanosomosis was not a major problem. All villages in this category were in the Savelugu District.

5 Conclusion

The study underscores the usefulness of cross-sectional studies as a precursor to tsetse and trypanosomosis control interventions. The results of the survey, besides identifying herds and villages where trypanosomosis constitutes a major problem, as far as animal health is concerned, also provided information on the prevalence of trypanosome species and strains as well as their vectors in the Savelugu and West Mamprusi Districts. The combined use of entomological, parasitological and serological methods in the survey provided more information than could have been obtained by any one method alone. The usefulness of the antibody indirect ELISA as a potential tool for the monitoring and evaluation of large-scale tsetse and trypanosomosis control interventions (Machila et al., 2001) was highlighted by the results of the survey.

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Legends of tables and figures

Table 1. Apparent density and infection rate in tsetse flies, and trypanosome challenge, in the Savelugu and West Mamprusi districts of Ghana

Table 2 : Occurrence of trypanosome species in cattle in the Savelugu and West Mamprusi districts of Ghana

Figure 1. Mean tsetse apparent density (number of tsetse flies caught per trap per day) in the Savelugu and West Mamprusi districts of Ghana

Figure 2. Comparison of parasitological and serological prevalence of bovine trypanosomosis at the village level in the West Mamprusi district

Figure 3. Trypanosomal antibody prevalence in age categories of cattle in the Savelugu and West Mamprusi districts

Figure 4. Prevalence of anaemia and of trypanosomosis in village cattle of the Savelugu and West Mamprusi districts

Figure 5. Prevalence of trypanosomoses and the prevalence of anaemia in the Savelugu and West Mamprusi Districts

437 Table 1. Apparent density and infection rate in tsetse flies, and trypanosome challenge, in the Savelugu and West Mamprusi districts of Ghana

DISTRICT	Number of tsetse flies dissected	Mean A.D[1] per site	Infection rate (%)	Challenge[2] index	Species of trypanosomes detected[3] (%)		
					T. vivax	*T. congolense* (savannah type)	*T. brucei*
Savelugu	77	1.8 (n=5)	2.6	4.7	0	50	50
West Mamprusi	168	8.6 (n=8)	2.3	19.8	50	25	25

438

439 1 A.D : Apparent Density (Mean number of tsetse per trap per day)

440 2 Challenge index = A.D. x Infection rate

441 3 Trypanosome species were identified by the PCR technique

442 Table 2: Occurrence of trypanosome species in cattle in the Savelugu and West Mamprusi districts of Ghana

443

District	Percentage of cattle infected	Occurrence of trypanosome species (%)					
		T. vivax	*T. congolense* (savannah)	*T. brucei*	TV and TC	TV and TB	TB and TC
Savelugu	8	66	17	12	1	2	2
West Mamprusi	16	80	9	3	6	1	1

444

445 TV : *T. vivax* TC :*T. congolense* TB : *T. brucei* .

446 Trypanosome species were identified by BCT and PCR

447

448
449

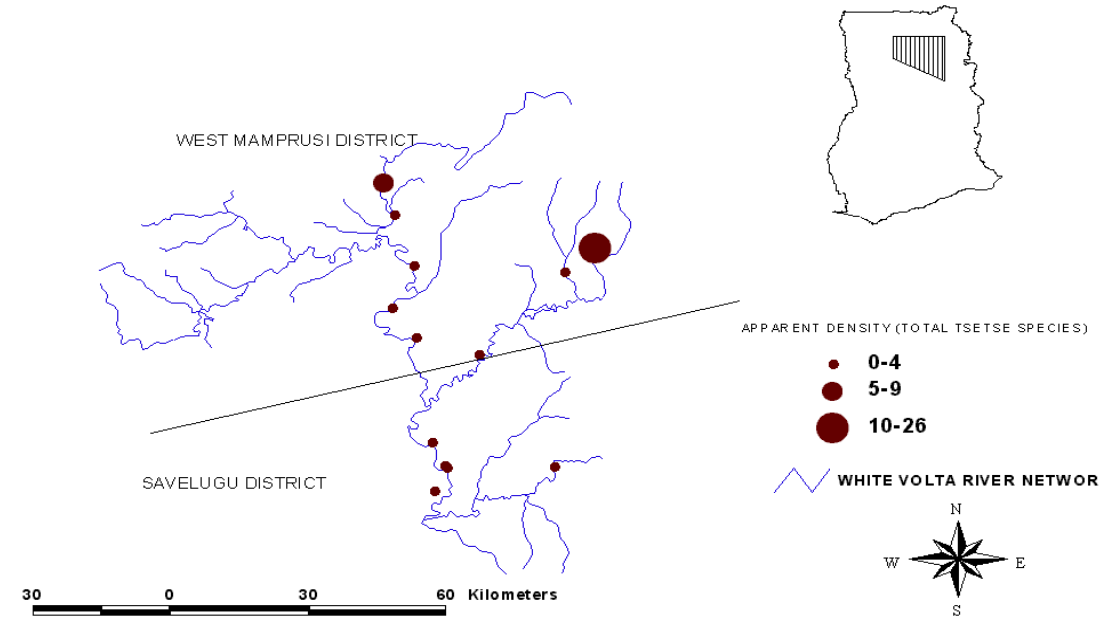
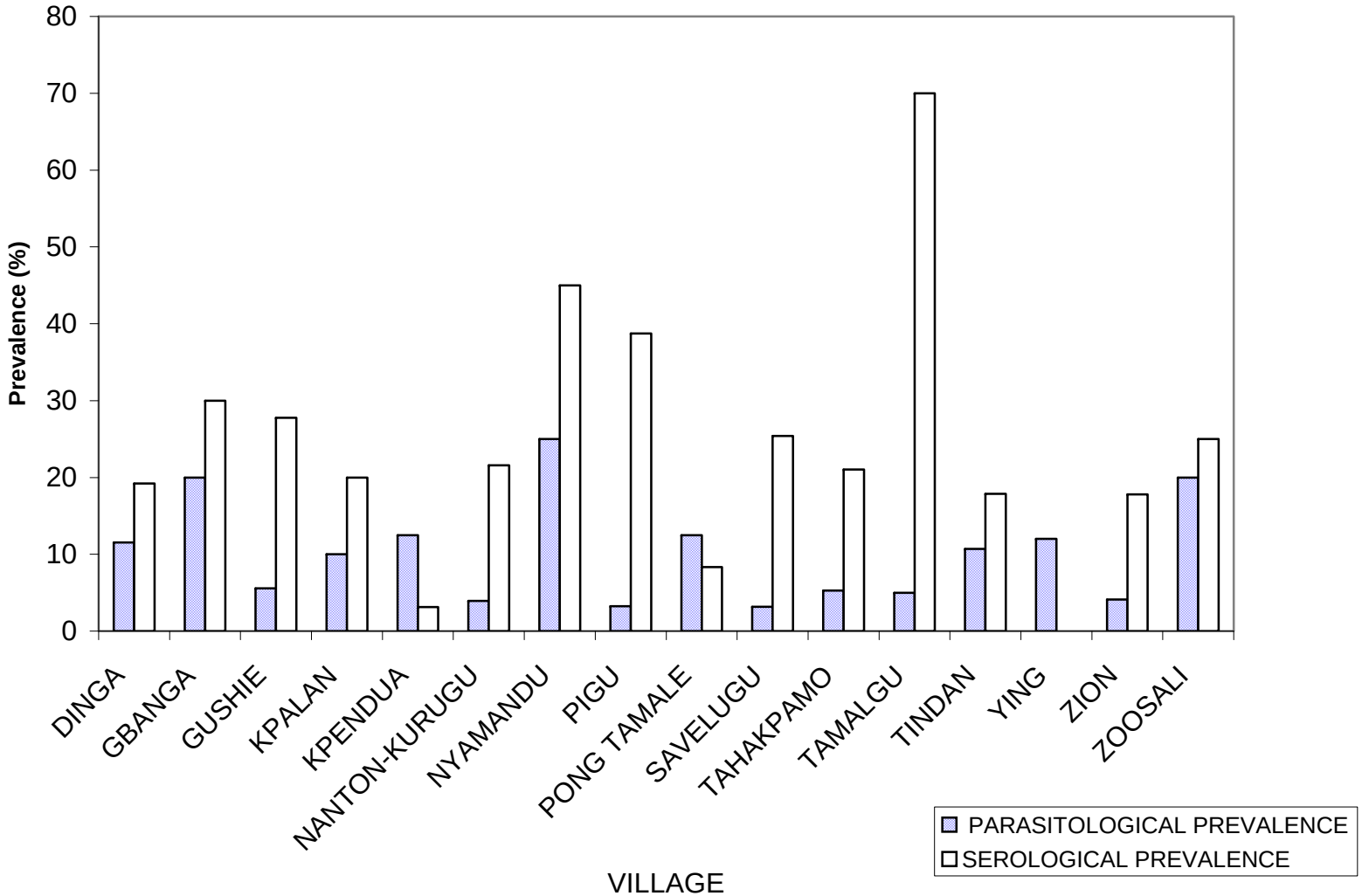
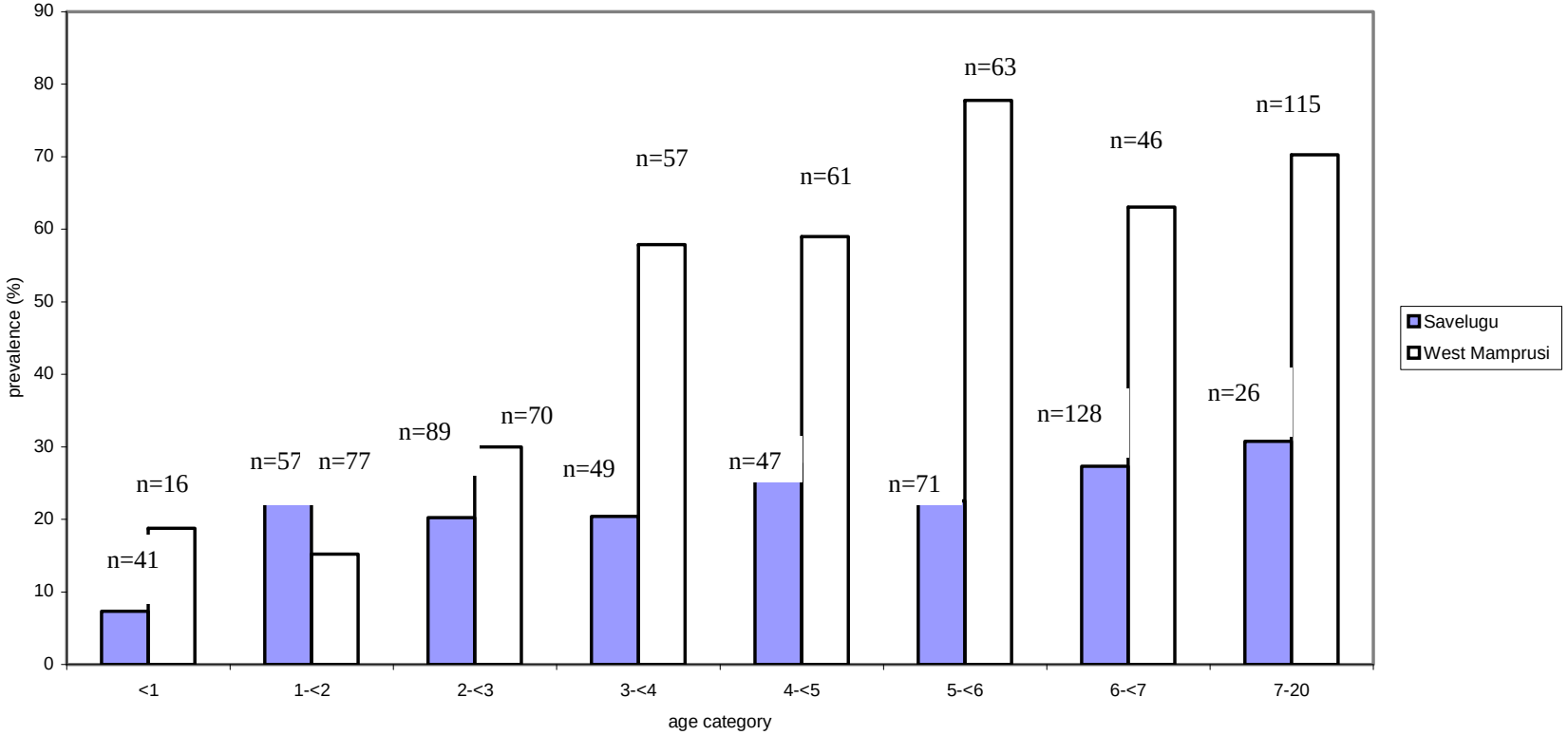


Fig. 1 Tsetse apparent density (number of tsetse caught per trap per day) in the Savelugu and West Mamprusi Districts


80
70
60
50
40
30
20
10
0
Prevalence (%)
DINGA
GBANGA
GUSHIE
KPALAN
KPENDUA
NANTON-KURUGU
NYAMANDU
PIGU
PONG TAMALE
SAVELUGU
TAHAKPAMO
TAMALGU
TINDAN
YING
ZION
ZOOSALI
PARASITOLOGICAL PREVALENCE
SEROLOGICAL PREVALENCE
VILLAGE

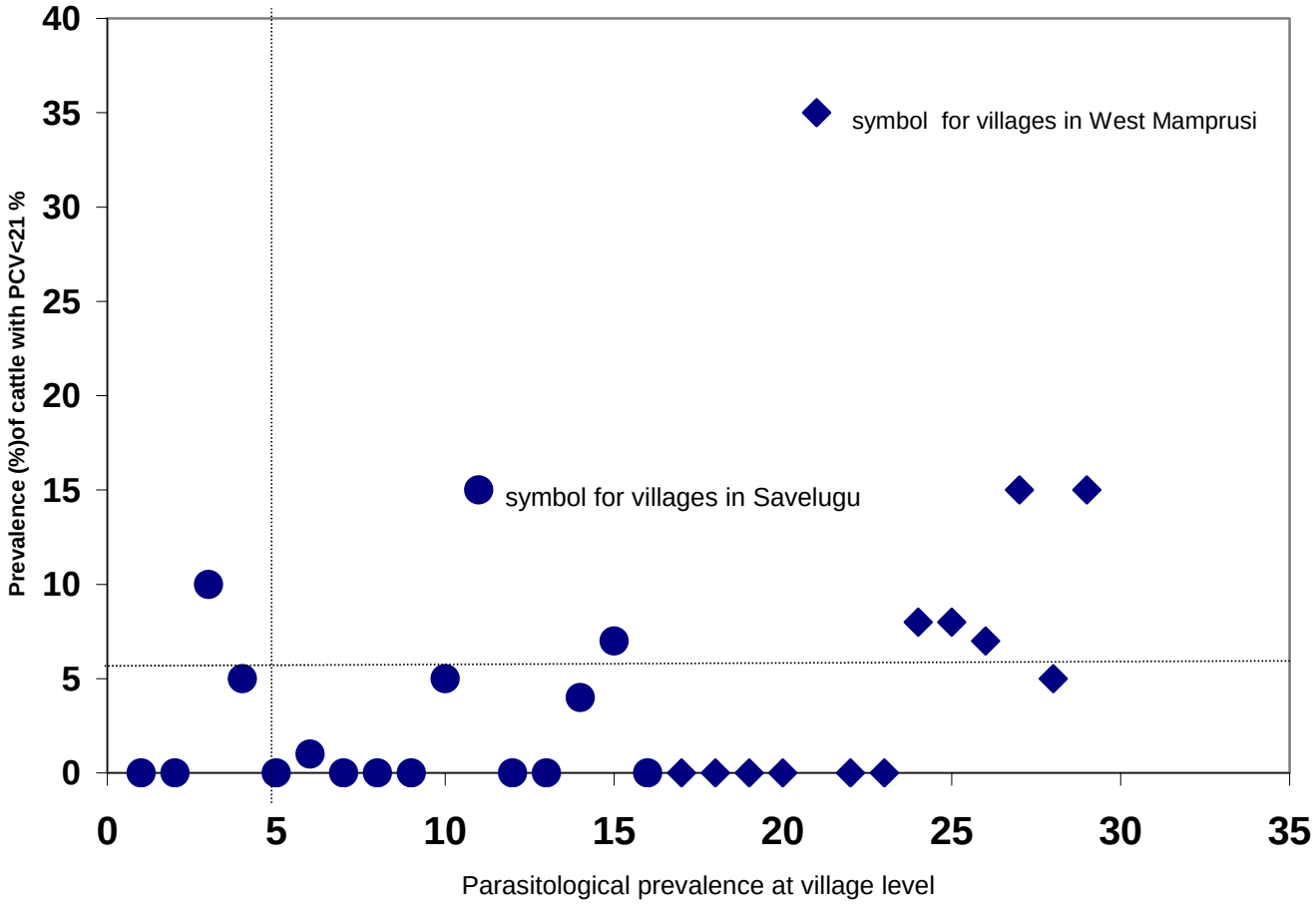
450

451


90
80
70
60
50
40
30
20
10
0
prevalence (%)
n=41
n=16
n=57
n=77
n=89
n=70
n=49
n=57
n=47
n=61
n=71
n=63
n=128
n=46
n=26
n=115
<1
1-<2
2-<3
3-<4
4-<5
5-<6
6-<7
7-20
age category
Savelugu
West Mamprusi

452

453


Prevalence (%)of cattle with PCV<21 %
40
35
30
25
20
15
10
5
0
0
5
10
15
20
25
30
35
symbol for villages in West Mamprusi
symbol for villages in Savelugu
Parasitological prevalence at village level

454

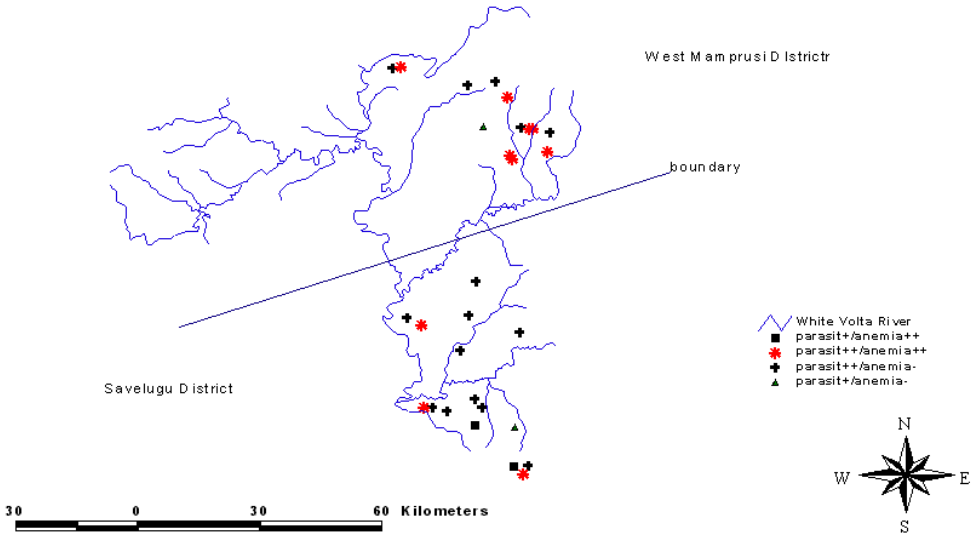


Fig 5. Prevalence of trypanosomosis and the prevalenceo of anaemia in the Savelugu and West Mamprusi Districts

455