

## The resurgence of trypanosomosis in Botswana

S P Sharma<sup>a</sup>, T C Losh<sup>a</sup>, M Malau<sup>b</sup>, K G Mangate<sup>a</sup>, K B Linchwe<sup>a</sup>, W Amanfu<sup>a</sup> and T K Motsu<sup>b</sup>

### ABSTRACT

No sleeping sickness or nagana cases have been reported in Botswana since 1985. In view of several confirmed clinical cases of nagana and reports of heavy bovine mortality, a parasitological survey was conducted to determine the prevalence of trypanosome infection in cattle in Maun and Shakawe areas of Ngamiland district. Wet blood films, buffy coat and Giemsa-stained thick and thin blood smears were used to detect trypanosomes in animals. Overall, trypanosome infection rate was 15.98%, with 5.94% and 27.29% in Maun and Shakawe respectively. The urgent need to combat trypanosomosis in Ngamiland, particularly in the Shakawe area, is highlighted, and a 3-phase integrated tsetse control strategy for this disease problem is discussed.

**Key words:** anaemia, Botswana, cattle, haematocrit, nagana, Ngamiland, tsetse fly resurgence, trypanosomosis.

Sharma S P, Losh T C, Malau M, Mangate K G, Linchwe K B, Amanfu W, Motsu T K **The resurgence of trypanosomosis in Botswana.** *Journal of the South African Veterinary Association* (2001) 72(4): 232–234 (En.). National Veterinary Laboratory, Private Bag 0035, Gaborone, Botswana.

African trypanosomosis, commonly known as nagana in animals and sleeping sickness in humans, continues to be a serious threat in over 10 million km<sup>2</sup> of sub-Saharan Africa. In southern Africa, trypanosomosis is also a significant animal health problem that has a profound impact on sustainable rural development and economic growth. The disease is transmitted between animals mainly by blood-sucking tsetse flies (*Glossina morsitans centralis*), which are confined to the Okavango Delta and the associated Kwando/Linyati River system in the Ngamiland and Chobe districts of Botswana.

Neither sleeping sickness nor nagana has been reported in Botswana since 1985. This is largely due to concerted efforts to eradicate tsetse flies by the Tsetse Control Division (TCD) of the Department of Animal Health and Production. In November 1999 and the first quarter of the year 2000, several clinical cases of nagana among cattle from Ngamiland were confirmed at the National Veterinary Laboratory in Gaborone. Recent reports from TCD have also indicated the extension of the tsetse fly range beyond originally known areas. In view of this, a survey was conducted to determine the prevalence of trypanosomosis in animals,

particularly cattle, in the Maun and Shakawe areas of the Ngamiland district.

A parasitological survey was conducted from April to June 2000 in the Ngamiland district as a result of farmers' complaints of cattle deaths and the diagnosis of trypanosomosis in parts of the district. During this survey, 1824 animals, comprising 1809 cattle, 10 goats and 5 donkeys of various ages and both sexes from 49 crush-pens, were screened for the presence of trypanosome parasites in the blood. Details of veterinary districts, extension areas and cattle crush-pens sampled are presented in Fig. 1. Animals were selected randomly from different crush-pens. Blood samples were collected in vacutainers, with and without anticoagulant, from the ear and jugular veins of animals for parasitological and serological examination. Parasitological studies included examination of wet blood films, determination of microhaematocrit (HCT) values, and examination of buffy coat, thick, and thin blood smears after staining with Giemsa, following standard laboratory techniques. The data were analysed statistically using standard statistics, namely mean, proportions, and standard error (SE), and Chi-square tests for comparison of proportions<sup>17</sup>.

Trypanosomes were observed in blood samples of 289 of 1809 cattle representing an infection rate of 15.98 ± 0.86 (SE) per cent in Ngamiland. In total, 57 of 959 and 232 of 850 cattle were positive for trypanosomes, indicating infection rates of 5.94 ±

0.76 (SE) and 27.29 ± 1.52 (SE) per cent in Maun and Shakawe respectively. The infection rate was significantly higher in Shakawe than Maun ( $\chi^2$  151.4,  $P < 0.01$ ). The greatest numbers of infected animals were detected in the Gumare and Seronga extension areas of Maun and Shakawe respectively. None of the goats and donkeys tested positive for trypanosomes.

The overall infection rates between males and females among bovines were almost identical. Of 507 young (< 18 months) and 1302 adult cattle sampled, 53 and 236 were positive, demonstrating respective infection rates of 10.45 ± 1.36 (SE) and 18.13 ± 1.07 (SE) percent. Our observations are in agreement with several previous studies<sup>7,8,19</sup>.

Comparing the different parasitological techniques used in the survey, the examination of buffy coat proved to be the most sensitive, detecting 82.5% of the positive animals followed by wet blood film (60.5%), thick smears (49.1%) and thin smears (43.6%), an observation that is in agreement with other reports<sup>3,6,16</sup>. In our opinion, the buffy coat technique is more useful because haematocrit values could also be recorded for individual animals and herd after centrifugation. At the herd level, the haematocrit profile or the herd average packed cell volume is considered a useful indicator of trypanosome infection and herd health. Owing to clotting and/or haemolysis of 11 blood samples, wet blood films and buffy coats could not be examined. However, thick and thin blood smears of 7 such animals were examined microscopically. Trypanosomes were detected in 74 animals by using all 4 techniques, whereas 50, 70 and 95 animals were diagnosed positive by 3, 2, and 1 of the laboratory techniques respectively. Although direct demonstration of trypanosomes in the infected animals by the techniques used in this investigation provided conclusive proof of infection, these techniques have been reported not to be as sensitive as some of the recently-introduced techniques such as AG-ELISA and polymerase chain reaction (PCR)<sup>18</sup>. Parasitological techniques cannot be used to detect latent trypanosome infection as most of the field infections are not associated with patent parasitaemia<sup>14</sup>. New technologies such as

<sup>a</sup>National Veterinary Laboratory, Private Bag 0035, Gaborone, Botswana.

<sup>b</sup>Tsetse Control Division, Department of Animal Health and Production, Private Bag 0014, Maun, Botswana.

Received: January 2001. Accepted: September 2001.

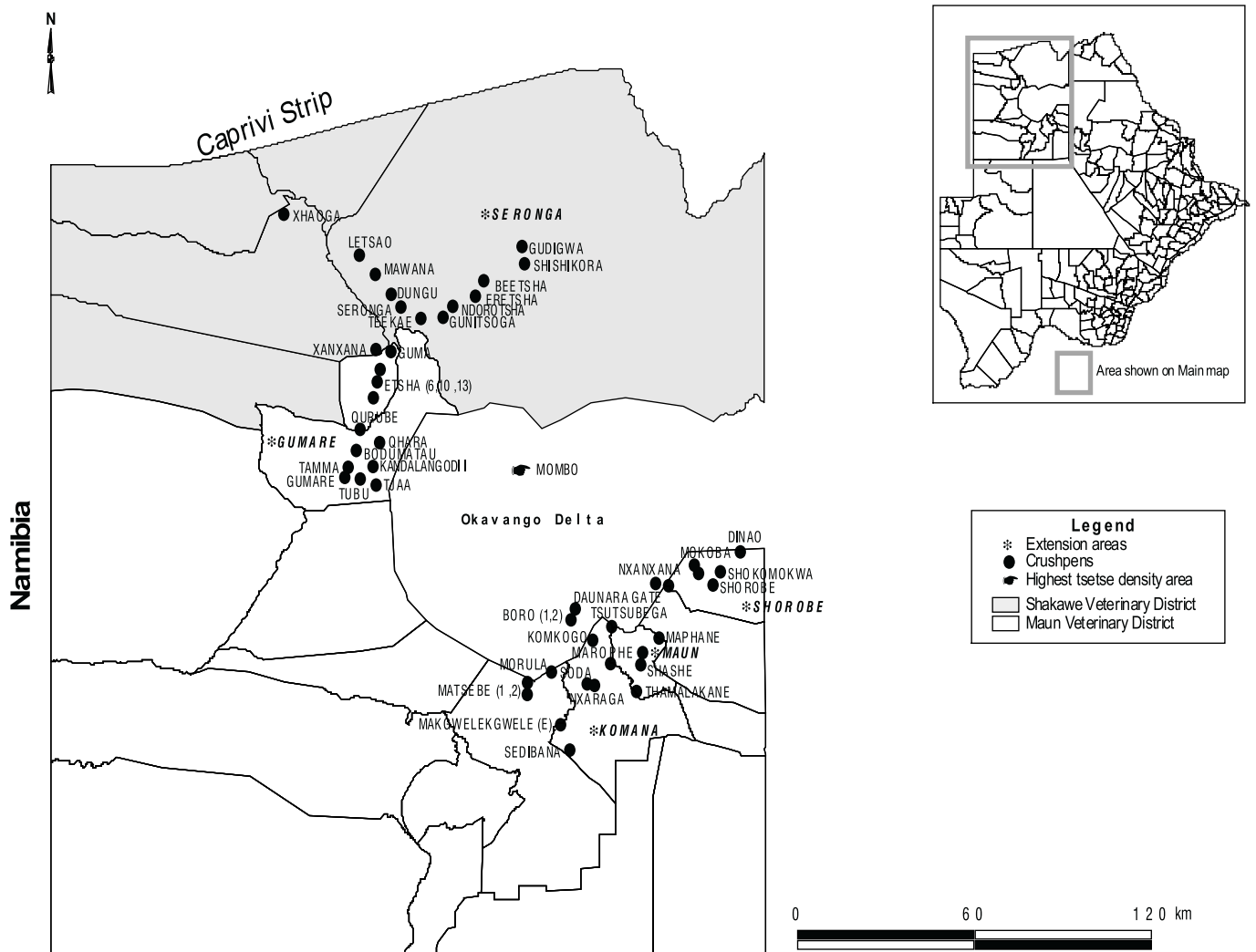


Fig. 1: Veterinary districts, extension areas and crushpens in Ngamiland covered during trypanosomosis survey.

AG-ELISA and PCR, with some improvement, are likely to revolutionise the diagnosis of trypanosomosis in animals, humans and tsetse flies<sup>10</sup>. However, according to a report, the sensitivity of parasitological and PCR techniques was approximately the same in detecting *Trypanosoma vivax* infection in cattle<sup>5</sup>.

Mean per cent haematocrit values of trypanosomosis-positive and negative cases in Maun were  $20.87 \pm 0.16$  (SE) and  $29.95 \pm 0.18$  (SE) respectively. In Shakawe, these were  $21.17 \pm 0.16$  (SE) and  $30.85 \pm 0.24$  (SE) in positive and negative cases respectively. There was a significant reduction ( $P < 0.05$ ) in the haematocrit values in the trypanosome-affected animals that corresponds with the observations of others<sup>1,2,9,15</sup>. The decrease in the haematocrit values indicating anaemia in animals with trypanosomosis is reported to be due to increased breakdown of erythrocytes during the development of parasitaemia<sup>11,13</sup>. Several mechanisms have been described as responsible for the destruction of erythrocytes, which include haemolysins and

enzymes, fever, complement and trypanosomal antigen produced by trypanosomes<sup>12,13</sup>.

Morphologically, trypanosome parasites identified during this survey were predominantly *Trypanosoma brucei* (52.5%), followed by *Trypanosoma congolense* (36.2%) and *Trypanosoma vivax* (11.3%). We did not attempt to differentiate between human and animal subspecies of *T. brucei*. More research is needed to identify trypanosome species using available molecular markers and isoenzyme characteristics.

Some animals suspected of trypanosomosis in 16 cattle crush-pens had reportedly received treatment with diminazene aceturate before this survey. The crushpens were Shashe, Maphane, Komkogo, Maun West, Matsebe I, Kandalangodi, Etsha 6, Tubu and Gumare in the Maun veterinary district and Seronga, Xhao, Gunitsoqa, Ndorotsha, Eretsha, Beetsha and Teekae in the Shakawe veterinary district. Berenil has been shown to have a limited prophylactic action against trypanosomosis<sup>4</sup>. It is difficult to know whether

the administration of diminazine aceturate to cattle might have influenced the prevalence rate of trypanosome infection recorded during this survey.

Based on the results of the present survey, an overall prevalence rate of 15.98% in Ngamiland and a significantly higher trypanosome infection rate of 27.29% in Shakawe in particular is a cause for concern. The Department of Animal Health and Production under the Ministry of Agriculture introduced an odour-bait technique (OBT) in 1992 after using annual aerial sprays for 20 years, in view of its popularity among Tsetse Control authorities throughout Africa. Resurgence of trypanosomosis can be attributed to a general upsurge of the tsetse population and expansion of the tsetse-infested area from 5000–7000 km<sup>2</sup> to 11 500 km<sup>2</sup>. This was probably due to failure to monitor and re-service approximately 25 000 deltamethrin-impregnated targets deployed around the Okavango Delta under OBT. These targets became inaccessible as a result of heavy floods in the delta and adjoining areas in 1999.

Lack of reliable and suitable transport also restricted access to targets. Apart from this, Ngamiland has a fairly naive cattle population after restocking a little over 3 years ago from nagana-free areas as a result of an outbreak of contagious bovine pleuropneumonia in the area. Exposure of such animals to a fairly large population of vector flies, besides stress of overcrowding and limited grazing areas on account of floods and heavy rains, might have exacerbated the severity of trypanosomiasis.

The Government of Botswana approved and implemented a 3-phased integrated tsetse control strategy in September, 2000 to avoid the recurrence of this disease. In the 1st phase curative and chemoprophylactic drugs such as diminazene aceturate and isometamidium chloride were used to treat all animals in endemic areas of Ngamiland district. The treatment was repeated twice because the prophylactic effects of these drugs are limited. It proved very effective in curtailing bovine mortality due to nagana. In the 2nd phase scheduled to start at the end of May 2001, a combination of OBT and aerial spraying with endosulfan will be put into practice for the next 2–3 years. Aerial sprays will cover highly-infected areas between Tubu/Etsha and Beetsha, and the area around Mombo with the highest tsetse density. The toxicity of endosulfan to young fish in shallow water as well as adverse effects of overdosing, overspraying and contamination of the environment, if any, will be monitored by a team of experts. The 3rd phase includes the sterile insect technique (SIT), whereby sterile male tsetse flies will be released across wide areas of the delta. SIT will be used if eradication of

tsetse is not achieved by OBT and aerial spraying.

#### ACKNOWLEDGEMENTS

We thank Dr M V Raborokgwe, Director, Department of Animal Health and Production, Ministry of Agriculture, Botswana, for granting permission to publish this paper, and officers at the Maun and Shakawe veterinary districts and the Tsetse Control Division for their assistance in the collection of blood samples.

#### REFERENCES

1. Abebe G, Jobre Y 1996 Trypanosomiasis: a threat to cattle production in Ethiopia. *Revue de Médecine Vétérinaire* 147: 897–902
2. Agyemang K, Dwinger R H, Little D A, Rowlands G J 1997 Village N'Dama cattle production in West Africa. Six years of research in the Gambia. International Livestock Research Institute (ILRI)/International Trypanotolerance Centre (ITC), Nairobi
3. Boyt, W P 1986 *Guide pratique pour le diagnostic, le traitement et la prévention de la trypanosomiase animale Africaine*. Food and Agricultural Organisation, Rome
4. Brander G C, Pugh D M, Bywater R J, Jenkins W L 1991 *Veterinary applied pharmacology and therapeutics* (5th edn). ELBS/Baillière Tindall, London
5. Desquesnes M 1997 Evaluation of a simple PCR technique for the diagnosis of *Trypanosoma vivax* infection in the serum of cattle in comparison to parasitological techniques and antigen-enzyme linked immunosorbent assay. *Acta Tropica* 65: 139–148
6. Desquesnes M, Tresse L 1996 Evaluation de la sensibilité du test de Wor pour la détection de *Trypanosoma vivax*. *Revue d'Élevage et de Médecine Vétérinaire des Pays tropicaux* 49: 315–321
7. Dwinger R H, Grieve A S, Snow F W, Rawlings P, Jabang B, Williams D J L 1992 Maternal antibodies in N' Dama cattle kept under trypanosomiasis risk in the Gambia. *Parasite Immunology* 14: 351–354
8. Fiennes R N T W 1970 Pathogenesis and

pathology of animal trypanosomiasis. In Mulligan H W (ed.) *The African trypanosomiasis*. George Allen and Unwin/Ministry of Overseas Development, London: 729–750

9. Griffin L, Allonby E W, Preston J W 1981 The interaction of *Trypanosoma congolense* and *Haemonchus contortus* infections in two breeds of goats. I. Parasitology. *Journal of Comparative Pathology* 91: 85–95
10. Masake R A 1997 Diagnosis of African trypanosomiasis (Review). In OAU/STRC, 1997: 69–77
11. Murray M, Dexter T M 1988 Anaemia in bovine African trypanosomiasis: a review. *Acta Tropica* 45: 389–432
12. Murray M, Morrison W I 1979 *Pathogenesis and pathology of African trypanosomiasis in domestic livestock*. Food and Agriculture Organisation, Rome: 14
13. Murray M, Nguyen H C, Lambert P H, Gerber H 1979 The anaemia of African trypanosomiasis. Demonstration of a haemolytic factor. 15th Meeting: International Scientific Council for Trypanosomiasis Research and Control (ISCTRC): 460–469
14. Nantulya V M, Diall O 1998 *Trypanosoma evansi* infections: towards penicillin diagnosis. *Journal of Protozoology Research* 8: 185–189
15. Otte M J, Abuabara J Y, Wells E A 1994 *Trypanosoma vivax* in Colombia: epidemiology and production losses. *Tropical Animal Health and Production* 26: 146–156
16. Paris J, Murray M, McOdimba F 1982 A comparative evaluation of the parasitological techniques currently available for the diagnosis of African trypanosomiasis in cattle. *Acta Tropica* 39: 307–316
17. Snedecor G W, Cochran W G 1967 *Statistical methods* (6th edn). Oxford and IBH Publishing, New Delhi
18. Solano P, Michel J F, Lefrançois T, Rocqué S de La, Sidibe I, Zoungrana A, Cuisance D 1999 Polymerase chain reaction as a diagnosis tool for detecting trypanosomes in naturally infected cattle in Burkino Faso. *Veterinary Parasitology* 86: 95–103
19. Whitelaw D D, Jordt T 1985 Colostral transfer of antibodies to *Trypanosoma brucei* in goats. *Annales de la Société Belge de Médecine Tropicale* 64: 199