ELSEVIER

Acta Tropica 78 (2001) 163–170

ACTA TROPICA

www.parasitology-online.com

The relationship between the parasitological prevalence of trypanosomal infections in cattle and herd average packed cell volume

P. Van den Bossche [a,*], G.J. Rowlands [b]

[a] *Institute of Tropical Medicine (ITM), Veterinary Department, Nationalestraat 155, Antwerpen, Belgium*
[b] *International Livestock Research Institute (ILRI), PO Box 307309, Nairobi, Kenya*

Received 19 May 2000; received in revised form 14 November 2000; accepted 24 November 2000

Abstract

The relationship between prevalence of trypanosomal infections (*Trypanosoma congolense*) and average packed cell volume (PCV) in herds of communally managed adult Angoni breed cattle was investigated in four districts of eastern Zambia. In all areas, regression analyses showed that the herd average PCV decreased with increasing prevalence of trypanosomal infections. The slope of the equation between average PCV and trypanosome prevalence decreased with increasing prevalence of trypanosomal infections. For the same increase in prevalence of trypanosomal infection, the decrease in herd PCV was higher in the areas with low to medium prevalence. Season of sampling also determined the slope of the regression equation. For the same increase in prevalence of trypanosomal infection, the decrease in herd PCV was higher during the dry compared to the rainy season suggesting that trypanosomosis is less well tolerated during the dry season. Results from the study suggest that the relationship between the prevalence of trypanosomal infections and herd average PCV could be a useful tool in the management of trypanosomosis and planning of its control. Reasons for the spatial and temporal variations in the relationship are discussed. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Bovine trypanosomosis; Prevalence; Packed cell volume; Disease impact

1. Introduction

The distribution of tsetse-transmitted bovine trypanosomosis is established usually by demonstrating trypanosomal infections in cattle. By comparing the proportion of infected animals between herds, areas of low, medium or high disease prevalence can be distinguished from disease-free areas. Several factors including; trypanosome species, trypanosome strain, age of the infected animal, breed and nutritional status affect the tolerance of trypanosomal infections in susceptible breeds (Connor, 1994). Therefore, decisions to intervene and control trypanosomosis cannot be based solely on the presence of trypanosome-in-

* Corresponding author. Tel.: + 32-3-2476396; fax: + 32-3-2476268.
E-mail address: pvdbossche@itg.be (P. Van den Bossche).

PII: S0001-706X(00)00182-0

fected animals but should be supported by an assessment of the impact of the infection on animal condition and, hence, animal production. Anaemia is a well-recognized and inevitable consequence of an infection with pathogenic trypanosomes in cattle and other domestic animals (Murray and Dexter, 1988). It is most commonly evaluated by determining the packed cell volume (PCV). In the absence of other factors causing anaemia the PCV usually gives an indication of the disease status of a trypanosome-infected animal and is correlated with its performance (Trail et al., 1991, 1993). Consequently, the herd average PCV should give a good indication of the health status of a herd. Furthermore, by establishing the relationship between herd average PCV and prevalence of infection in an area, useful information can be obtained on: (1) the impact of varying levels of disease prevalence on herd health; and (2) the likely impact of control interventions on herd health. Although the herd average PCV is expected to decrease with increasing prevalence of trypanosomosis, this relationship has not been quantified sufficiently. Data obtained from trypanosomosis surveys conducted in eastern Zambia were used to study this relationship in more detail. The usefulness of this relationship as an adjunct to the rapid assessment of the impact of bovine trypanosomosis is discussed.

2. Materials and methods

The herd average prevalence of trypanosomal infections and herd average PCV were obtained from cattle sampled at 141 sampling sites (crush-pens) in Katete, Petauke, Chipata and Lundazi districts of eastern Zambia. All districts are ecologically similar, have similar farming systems (Doran, 1998, 1999), and are situated on the eastern plateau. Bovine trypanosomosis, transmitted by *Glossina morsitans morsitans* Westwood, is endemic. Bovine trypanosomosis is managed using trypanocidal drugs, mainly diminazene aceturate (Berenil®, Hoechst). The proportion of animals treated and the treatment frequency are low (Van den Bossche et al., 2000). Two separate surveys were conducted. The first survey covered all four districts. It was conducted during the rainy season (November 1995–March 1996). Sampling sites were selected depending on their location and were evenly spread over each district. During a second survey, sampling was repeated at the sampling sites in Petauke district only but different animals were sampled. The second survey was conducted during the dry season (August–September, 1996).

A random sample of communally managed adult cattle (Angoni breed) was selected at each sampling site. The size of the sample at each sampling site was calculated according to Cannon and Roe (1982). It depended on the total cattle population at a particular sampling site but never exceeded 60 head of cattle. The buffy coat, stained thick and stained thin smear were used as parasitological diagnostic tests (Paris et al., 1982). 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 9000 rpm. After centrifugation, the packed cell volume (PCV) was determined. 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 40 × objective lens. Giemsa-stained thick and thin blood smears were examined under 100 × oil immersion objective lens for trypanosome species identification.

The level of bovine trypanosomosis at a sampling site was calculated as the proportion of cattle with a trypanosomal infection. Since the majority of trypanosomal infections in cattle in the survey area were due to *Trypanosoma congolense*, the disease prevalence figures only refer to infections with this trypanosome species. Cattle with *T. vivax* and *T. brucei infections* were discarded from the analysis because of the very low prevalence of both trypanosome species. The PCV of all animals sampled at each site was averaged; this is referred to as the ‘herd average PCV’.

From all animals, blood contained in one heparinized microhaematocrit centrifuge capillary tube was extruded onto a filter paper (Whatman no. 4, Whatman®). Eluted blood spots were screened for the presence of trypanosomal antibodies using an indirect ELISA (Hopkins et al., 1998). Data on the prevalence of cattle with anti-trypanosomal antibodies were used to compare parasitological-negative/serological-negative with parasitological-negative/serological-positive herds.

The relationship between the parasitological prevalence of trypanosomal infections and herd average PCV was examined by regression analysis using the estimates of herd average PCV as the dependent variable and the prevalence of trypanosomal infections in a herd as the independent variable. The effects of area of sampling (district) and season of sampling on the herd average PCV were also investigated by analysis of variance using data obtained from the four districts in Zambia during the rainy and one district during the dry season. In addition, use was made of a multiple linear regression model with herd average PCV as the dependent variable and combinations of season, area of sampling (district) and herd prevalence of trypanosomal infections as independent variables.

The models fitted were:

$y_{ij} = a + d_i + b_i x_{ij} + r_{ij}$ for all four districts

($i = 1, \ldots, 4$) during the rainy season; and

$y_{ij} = a + s_i + b_i x_{ij} + r_{ij}$ for rainy and dry season

($i = 1, 2$) for herds in Petauke district,

where: y_{ij} = herd average PCV for herd j within respectively district or season i; a, average intercept; d_i ($i = 1, \ldots, 4$), effect of district on average herd PCV; s_i ($i = 1, 2$), effect of season on average herd PCV; b_i, regression coefficient for district in season i; x_{ij}, prevalence of trypanosomal infections in a herd; and r_{ij}, residual. All analyses were performed using the statistical package SPSS (SPSS Inc.).

3. Results

A total of 8640 head of cattle were sampled in four districts (Chipata, Lundazi, Petauke and Katete) of the Eastern Province of Zambia. Trypanosomal infections, mainly due to *T. congolense* (86.4%), were detected in 1252 animals (14.5%) from 97 herds out of a total of 141 herds sampled.

The herd average prevalence of trypanosomal infections in the rainy season varied between areas. It was highest in Petauke and Lundazi districts and lowest in Chipata district (Table 1). The average PCV of parasitologically positive herds (*i.e.* herds with at least one animal that was found to be infected with trypanosomes) decreased with increasing average prevalence of infection. The majority of the parasitological negative herds (68.4%) contained animals with anti-trypanoso-

Table 1
Number of herds sampled, number of trypanosome-positive herds, average parasitological prevalence of trypanosomosis and average PCV of parasitological positive and negative herds in four districts of the Eastern Province of Zambia during the rainy season and in Petauke District during the dry season

Area	Season	Number of herds sampled	Number of positive herds	Average prevalence (% ± 1 S.E.)	Average PCV (% ± 1 S.E.)	
					Positive herds	Negative herds
Chipata	Rain	21	10	8.4 ± 2.3	29.9 ± 0.9	31.8 ± 0.8
Katete	Rain	16	13	11.4 ± 1.6	27.7 ± 0.7	29.7 ± 0.8
Lundazi	Rain	22	22	17.8 ± 1.8	27.1 ± 0.5	–
Petauke	Rain	43	38	24.7 ± 2.8	26.5 ± 0.3	29.6 ± 0.9
Petauke	Dry	39	39	26.1 ± 4.2	26.1 ± 0.5	–

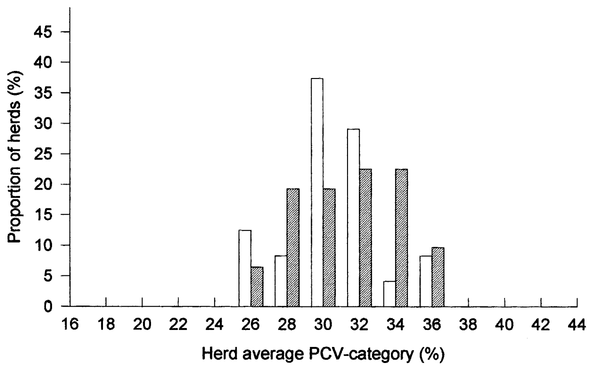

Fig. 1. Frequency distribution of average packed cell volume (PCV) of parasitologically negative herds that are serologically negative ($n=6$) (□) and serologically positive ($n=13$) (▥).

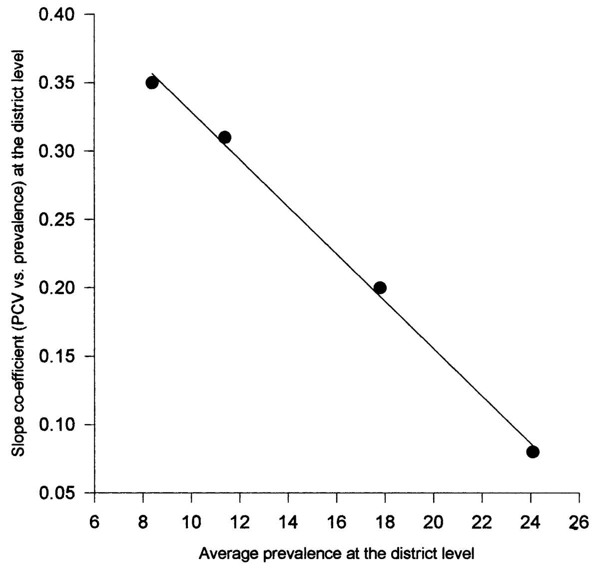

Fig. 2. Linear regression of slope-coefficients against average prevalence of trypanosomal infections at the district level.

mal antibodies. However, the herd average PCV of parasitologically negative and seronegative herds (31.8 ± 1.2%, $n=6$) did not differ from the herd average PCV of parasitologically negative but seropositive herd (30.5 ± 0.5%, $n=13$) (Fig. 1).

In all districts, regression analyses showed that the herd average PCV of parasitologically positive herds decreased with increasing prevalence of trypanosomal infections (Table 2). Removal of district from the multiple linear regression model did not result in a significant change in its fit ($P>0.05$). Hence, the intercepts for area of sampling shown in Table 2 were not significantly different. The slope of the equation between average PCV and trypanosome prevalence (parameter b in Table 2), however, decreased with increasing prevalence of trypanosomal infections at the district level suggesting a curvilinear relationship with PCV (Figs. 2 and 3). Season of sampling also affected the slope of the equation between mean PCV and trypanosome prevalence (parameter b in Table 3). For the same increase in prevalence of infection the decrease in herd average PCV was higher in the dry compared to the rainy season ($P<0.01$) (Table 3; Fig. 4).

4. Discussion

The development of anaemia is one of the most typical signs of trypanosomosis caused by *T. congolense* in susceptible cattle breeds (Murray and Dexter, 1988). The level of anaemia or the PCV usually gives a reliable indication of the disease status and productive performance of an infected

Table 2
Linear regression of herd average PCV (%) on herd prevalence of trypanosomal infections (%) in four districts of the Eastern Province of Zambia during the rainy season[a]

Area	Season	Number of herds	*a*	*b*	*r*	Significance
Chipata	Rainy	10	32.8 ± 0.8	−0.35 ± 0.08	0.84	$P<0.001$
Katete	Rainy	13	31.3 ± 1.3	−0.31 ± 0.11	0.65	$P<0.05$
Lundazi	Rainy	22	30.6 ± 0.9	−0.20 ± 0.04	0.71	$P<0.001$
Petauke	Rainy	38	28.4 ± 0.4	−0.08 ± 0.02	0.65	$P<0.001$

[a] *a*, intercept (±1 S.E.); *b*, regression coefficient (±1 S.E.); *r*, correlation coefficient.

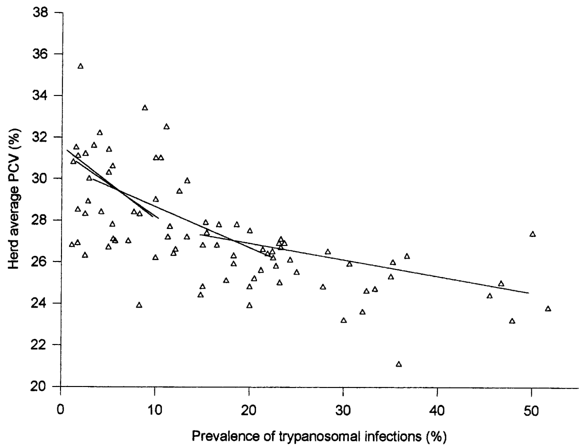

Fig. 3. Relationship between herd average PCV and prevalence of trypanosomal infections in herds sampled during the rainy season in eastern Zambia. Lines, referring to data grouped at the district level, are fitted by linear regression; see Table 2 for parameter estimates and significance levels.

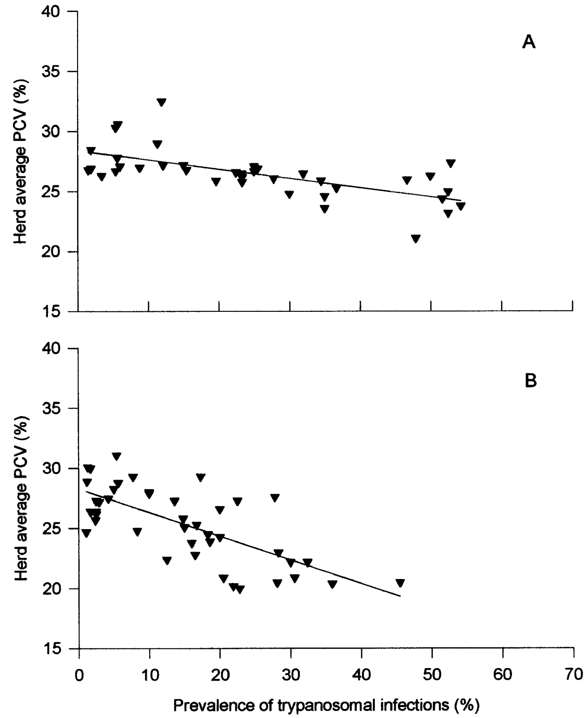

Fig. 4. Relationship between herd average PCV and parasitological prevalence of trypanosomal infections in herds sampled during the rainy (A) and dry (B) season in Petauke District. See Table 3 for parameter estimates and significance levels.

animal (Trail et al. 1991, 1993). Bovine trypanosomosis control aims at reducing the prevalence of infection with a concomitant increase in the herd average PCV (Bauer et al., 1999). Therefore, knowledge of the relationship between the prevalence of trypanosomal infections and herd average PCV could be a useful tool in the preliminary assessment of the expected impact of a control intervention. Conversely, the slope of the regression line can be used as an indicator of the impact of trypanosomosis on herd average PCV and, hence, herd health. By comparing the slopes, temporal and spatial comparisons could be made of the impact of trypanosomosis. However, the herd average PCV is affected by factors other than trypanosomosis (Connor, 1994). These confounding factors are not always easily identifiable but they are likely to affect both trypanosomosis positive and negative animals. Hence, the average PCVs of trypanosomosis-free herds are a good indicator of the levels of anaemia in the absence of trypanosomosis and could form the baseline for comparison between areas. Determining the PCV-distribution of parasitologically negative herds may be difficult with parasitological diagnostic methods of low sensitivity at the individual animal level (Paris et al., 1982). However, according to the above results, the herd-level sensitivity of the buffy coat method for the detection of at

Table 3
Linear regression of herd average PCV (%) on herd prevalence of trypanosomal infections (%) in Petauke district of the Eastern Province of Zambia during the rainy and dry season[a]

Area	Season	Number of herds	*a*	*b*	*r*	Significance
Petauke	Rainy	38	28.4 ± 0.4	-0.08 ± 0.02	0.65	$P<0.001$
Petauke	Dry	39	28.3 ± 0.6	-0.20 ± 0.03	0.71	$P<0.001$

[a] *a*, intercept ($\pm$1 S.E.); *b*, regression coefficient ($\pm$ 1 S.E.); *r*, correlation coefficient.

least one positive sample in a positive herd appears to be high in relation to the sample sizes used in the survey (Martin et al., 1992). Indeed, the herd average PCV of parasitologically negative but serologically positive herds does not differ from the herd average PCV of serologically negative herds. The PCV distribution of the parasitologically negative herds can thus be used as the baseline herd average PCV distribution in the absence of trypanosomosis.

The slope of the regression line between herd average PCV and herd trypanosome prevalence decreased with increasing prevalence of trypanosomal infections. Hence, for the same increase in trypanosome prevalence, the decrease in herd average PCV is lower in herds under high challenge compared with herds under low challenge. This could be explained by differences in trypanocidal drug use. However, results from a trypanocidal drug use survey conducted in all four survey areas, suggest that strategies in trypanocidal drug use do not differ much between the districts (Van den Bossche et al., 2000). In all areas, cattle owners mainly treat clinically sick animals.

For the same increase in prevalence of trypanosome infection, the decrease in herd PCV is higher in the areas with low to medium prevalence (Chipata, Katete and Lundazi districts). A primary factor determining the slope of the equation between PCV and trypanosome prevalence may be the infected animals' abilities to control the development of anaemia. This will differ between cattle breeds and will be affected by management-related factors, such as nutrition, and the level of acquired immunity or a combination of the two. The effect of cattle breeds is well-known. The development of anaemia tends to be more severe in exotic breeds whereas it is well-controlled in trypanotolerant breeds (d'Ieteren et al., 1998). All cattle sampled during the survey were of the trypanosusceptible Angoni breed. Moreover, livestock management practices do not differ between the survey areas (Doran, 1998, 1999). It has been shown that infection with one serodeme of trypanosome can induce immunity to reinfection with that particular serodeme but not to heterologous challenge. The immunity conferred, however, is short lived and is no longer apparent 6 months after the initial challenge (Murray and Urquhart, 1977). The development of such a non-sterile immunity to bovine trypanosomosis is difficult to assess but has been observed in areas of high challenge where curative drugs are used to treat the acutely sick animals (Hornby, 1941; Boyt, 1967; Wilson et al., 1976; Bourn and Scott, 1978). Such conditions do prevail in the Petauke district of eastern Zambia and may explain the observed difference in the relationship between the prevalence of infection and the herd average PCV in the Petauke district and in the other four districts. Indeed, in Katete, Chipata and, to a lesser extent, Lundazi district, challenge is low and irregular. This level of challenge may be insufficient for the development of protective immunity in cattle resulting in higher decrease in PCV for the same increase in prevalence compared to cattle in the Petauke area. Finally, the proportion of acute and chronic trypanosomal infections may affect the relationship between herd average PCV and prevalence of trypanosomal infections in an area. Possible differences in the impact of chronic and acute infections on herd average PCV were not assessed in this study. It is, however, unlikely that the proportion of acute trypanosomal infections will differ greatly between trypanosomosis endemic areas.

In a study of the effect of a tsetse control intervention in Ethiopia using an insecticidal pour-on applied to village zebu cattle average trypanosome prevalences before and during tsetse control were 39.1 and 14.6%, respectively (Rowlands et al., 1999). Corresponding changes in PCV were from 24.3 to 25.9%. The slope, calculated as $(24.3-25.9)/(39.1-14.6) = -0.065$ is similar in magnitude to the slope predicted from the results at Petauke in the dry season (Table 2). The zebu cattle were maintained under high levels of trypanocidal drug resistance. Animals were bled monthly and any animals detected with trypanosomes and with a PCV $<$ 26% were treated with diminazene aceturate. The fact that cattle survived under this treatment regime, despite high levels of drug resistance, suggest that they had acquired some degree of protective immunity. In this respect the conditions of constant challenge prevailing at the site were similar to those at Petauke. In contrast, a study of the impact of the utilisation of biconical traps on the prevalence of trypanosomal infections in trypanotolerant and trypanotolerant x zebu village cattle in Côte d'Ivoire resulted in a reduction from 12.6 to 2.8% in prevalence (Rowlands et al., 1996). The corresponding change in PCV was from 29.8 to 32.2% resulting in a slope, as calculated above, of − 0.24.

The two studies differ in treatment regimes (only sick animals were treated in Côte d'Ivoire), breeds of cattle and environmental and management aspects. Nevertheless, the difference in the two slopes, when compared to the levels of trypanosome prevalence (39.1 and 12.6%, respectively) observed before onset of tsetse control, is in line with the inferences drawn from the present study in eastern Zambia.

A major factor affecting the PCV is the plane of nutrition. Poor nutrition is known to result in a lower PCV (Sawadogo et al., 1991; Katunguka-Rwakishaya et al., 1995). A study, investigating the effect of season on the food intake of Angoni cattle in eastern Zambia showed that poor pasture conditions and high temperatures cause nutritional stress during the dry and especially the hot dry season (De Clercq, 1997). Thus, the observed effect of season on the association between prevalence and herd average PCV is very likely to have been due to poor nutrition in the dry season. Whereas the season of sampling has no effect on the average PCV of the parasitologically negative herds, as indicated by the intercepts a in Table 3, trypanosomosis seems to be less well tolerated during the dry season. This may explain the high proportion of trypanocidal drug treatments administered during the dry season (Van den Bossche et al., 2000).

The above results on the relationship between the prevalence of trypanosomal infections and herd average PCV could be a useful tool in the management of trypanosomosis and planning of its control. Further research is recommended on the temporal and spatial variations of this relationship and the factors affecting it.

Acknowledgements

The work presented in this paper was undertaken in collaboration with the Department of Animal Production and Health (Zambia). The staff assisting with the survey are kindly acknowledged. The Central Veterinary Research Institute (Balmoral, Zambia) is kindly acknowledged for the assistance with the analyses of the ELISA samples. Drs R.J. Connor and P. Coleman are acknowledged for commenting on the manuscript. The work was funded by the sixth European Development fund through the Regional Tsetse and Trypanosomosis Control Programme (Accounting number 7.ACP.ZA.002 and 6.ACP.RPR.468).

References

Bauer, B., Amsler-Delafosse, S., Kaboré, I., Kamuanga, M., 1999. Improvement of cattle productivity through rapid alleviation of African Animal Trypanosomosis by integrated disease management practices in the agropastoral zone of Yalé, Burkina Faso. Trop. Anim. Hlth. Prod. 31, 89–102.

Bourn, D., Scott, M., 1978. The successful use of work oxen in agricultural development of tsetse infested land in Ethiopia. Trop. Anim. Hlth. Prod. 10, 191–203.

Boyt, W.P., 1967. The veterinary contribution to the control of trypanosomiasis. Proc. Trans. Rhod. Sci. Ass. 52, 16–20.

Cannon, R.M., Roe, R.T., 1982. Livestock disease surveys. A field manual for Veterinarians. Australian Government Publishing Service, Canberra, p. 26.

Connor, R.J., 1994. African animal trypanosomiases. In: J.A.W. Coetzer, G.R. Thomson, R.C. Tustin (Eds.), Infectious diseases of livestock with special reference to southern Africa, Oxford University Press, Cape Town, pp. 166–203.

Doran, M., 1998. Socio-economic surveys of tsetse-free and tsetse-infested areas of Eastern Province, Zambia, 1997. Regional Tsetse and Trypanosomiasis Control Programme, Harare, p. 42.

Doran, M., 1999. Socio-economic surveys of tsetse-free and tsetse-infested areas of Eastern Province, Zambia, 1998. Regional Tsetse and Trypanosomiasis Control Programme, Harare, p. 40.

De Clercq, K., 1997. Feeding evaluation of the Angoni cattle during the late dry season in Chipata (Zambia). MSc thesis, University Ghent, Ghent, p. 143.

d'Ieteren, G.M.D., Authié, E., Wissocq, N., Murray, M., 1998. Trypanotolerance, an option for sustainable livestock production in areas at risk of trypanosomosis. Rev. Sci. Tech. Off. Int. Epiz. 17, 154–175.

Hopkins, J.S., Chitambo, H., Machila, N., Luckins, A.G., Rae, P.F., Van den Bossche, P., Eisler, M.C., 1998. Adaptation and validation of the antibody trapping ELISA using dried blood spots on filter paper, for epidemiological surveys of tsetse transmitted trypanosomosis in cattle. Prev. Vet. Med. 37, 91–99.

Hornby, H.E., 1941. Immunisation against bovine trypanosomosis. Trans. R. Soc. Trop. Med. Hyg. 35, 165–176.

Katunguka-Rwakishaya, E., Parkins, J.J., Fishwick, G., Murray, M., Holmes, P.H., 1995. The influence of energy intake on the pathophysiology of *Trypanosoma congolense* infection in Scottish Blackface sheep. Vet. Parasitol. 59, 207–218.

Martin, S.W., Shourki, M., Thorburn, M.A., 1992. Evaluating the health status of herds based on tests applied to individuals. Prev. Vet. Med. 14, 33–43.

Murray, M., Urquhart, G.H., 1977. Immunoprophylaxis against African trypanosomiasis. In: L.H. Miller, J.A. Pino, J.J. McKelvey (Eds.), Immunity to blood parasites of animals and man. Plenum Press, London, pp. 209–241.

Murray, M., Dexter, T.M., 1988. Anaemia in bovine African Trypanosomiasis: a review. Acta Trop. 45, 389–432.

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 Trop. 39, 307–316.

Rowlands, G.J., d'Ieteren, G.D.M., Coulibaly, L., Hecker, P.A., Leak, S.G.A., Nagda, S.M., 1996. Assessment of the effect of tsetse control on livestock productivity — a case study in northern Côte d'Ivoire. Prev. Vet. Med. 28, 17–32.

Rowlands, G.J., Woudyalew Mulatu, Leak, S.G.A., Nagda, S.M. and d'Ieteren, G.D.M., 1999. Estimating the effects of tsetse control on livestock productivity — a case study in southwest Ethiopia. Trop. Anim. Hlth. Prod. 31, 279–294.

Sawadogo, G.J., Oumarou, A.A., Sene, M., Diop, M., 1991. Effect of poor pasture conditions and type of feeding on some biochemical values of Gobra Zebu in Senegal. Br. Vet. J. 147, 538–544.

Trail, J., D'Ieteren, G.D.M., Feron, A., Kakiese, O., Mulungo, M., Pelo, M., 1991. Effect of trypanosome infection, control of parasitaemia and control of anaemia development on productivity of N'Dama cattle. Acta Trop. 48, 37–45.

Trail, J.C.M., D'Ieteren, G.D.M., Murray, M., Ordner, G., Yangari, G., Maille, J.C., Viviani, P., Colardelle, C., Sauveroche, B., 1993. Measurements of trypanotolerance criteria and their effect on reproductive performance of N'Dama cattle. Vet. Parasitol. 45, 241–255.

Van den Bossche, P., Doran, M., Connor, R.J., 2000. An analysis of trypanocidal drug use in the Eastern Province of Zambia. Acta Trop. 75, 247–258.

Wilson, A.J., Paris, J., Luckins, A.G., Dar, F.K., Gray, A.R., 1976. Observations on a herd of beef cattle maintained in a tsetse area II. Assessment of the development of immunity in association with trypanocidal drug treatment. Trop. Anim. Hlth. Prod. 8, 1–12.