

Detection of antibodies to the *Ehrlichia ruminantium* MAP1-B antigen in goat sera from three communal land areas of Zimbabwe by an indirect enzyme-linked immunosorbent assay

O. KAKONO¹, T. HOVE¹, D. GEYSEN² and S. MAHAN³

ABSTRACT

KAKONO, O., HOVE, T., GEYSEN, D. & MAHAN, S. 2003. Detection of antibodies to the *Ehrlichia ruminantium* MAP1-B antigen in goat sera from three communal land areas of Zimbabwe by an indirect enzyme-linked immunosorbent assay. *Onderstepoort Journal of Veterinary Research*, 70:243–249

A total of 1 286 caprine serum samples collected from three communal land areas in Zimbabwe from March 1999 to February 2000 were tested for *Ehrlichia ruminantium* antibodies using the indirect MAP1-B enzyme-linked immunosorbent assay. Of the 480 samples tested from Mudzi, a non-heartwater area, 425 (89.4 %) were positive. In the heartwater endemic areas, of the 441 samples 352 (79.4 %) from Gwanda and 300 of the 365 samples (83.2 %) from Bikita tested positive. The seroprevalence in the Bikita and Gwanda (approaching 90 %) is consistent with reports in related serological surveys that puts the seroprevalence of *E. ruminantium* in goats from endemic areas of Zimbabwe at 90 %. However, the high seroprevalence in the non-heartwater area of Mudzi is unexpected and can be a result of the presence of a serologically cross-reacting organism, which has to be isolated and characterized. The results need to be confirmed by alternative tests, based on molecular diagnostic tools. There were no significant differences in seroprevalence between the three sampling areas as there were between the three sampling periods. The highest corresponded with the period January to February (peak tick activity) and the lowest with the period July to September (minimal tick activity).

Keywords: Antibodies, caprine, *Ehrlichia ruminantium*, ELISA, heartwater, MAP1-B, serological responses

INTRODUCTION

Heartwater is a tick-borne disease of cattle, sheep, goats and some wild ruminants caused by the rickettsia *Ehrlichia ruminantium* (Camus & Barre 1988; Morel 1989; Bezuidenhout, Prozesky, Du Plessis & Van Amstel 1994; Dumler, Barbet, Bekker, Dasch, Palmer & Ray 2001). The main vectors of the dis-

ease in southern Africa are *Amblyomma hebraeum* and *Amblyomma variegatum* (Norval 1994).

The disease is of considerable economic importance in sheep and goats (Yunker 1996). It is one of the major constraints on the health of small stock in endemic areas and makes upgrading of indigenous stock difficult (Bezuidenhout *et al.* 1994). Camus & Barré (1988) believe that small ruminants are more susceptible to heartwater than all the other ruminants. The epidemiology of heartwater in small ruminants is poorly understood as most information relates to cattle that are the preferred hosts of adult *Amblyomma* ticks, whereas goats are predominantly parasitized by the immature stages that would result in different transmission patterns (Yunker 1996). Heartwater has been implicated as the

¹ Faculty of Veterinary Science, P.O. Box M.P. 167, Mount Pleasant, Harare, Zimbabwe

² Prince Leopold Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerp, Belgium

³ University of Florida/USAID/SADC Heartwater Research Project, P.O. Box CY551, Causeway, Harare, Zimbabwe

leading killer of kids in Mozambique (Asselbergs, Jongejan, Langa, Neves & Afonso 1993) and adult goats in Zimbabwe (CARD/GOZ 1993). The prevalence of the disease in goats is actually underestimated because the disease is difficult to diagnose as the usual presenting sign is sudden death. In Zimbabwe it is estimated that 30% of goat mortality is due to heartwater (CARD/GOZ 1993).

Ninety-seven per cent of goats in Zimbabwe are found in the communal land and resettlement areas (Kusina & Kusina 1998), particularly in the low-lying areas of the Zambezi, Save and Limpopo valleys (< 900 m in altitude). In these areas goats play a prominent role in the provision of meat (protein source), cash, manure and skins (Chezhira 1993; Kusina & Kusina 1998). They also play important roles in cultural and social functions and are a source of milk in certain parts of the country (Kusina & Kusina 1998). Despite this immense contribution to the life of subsistence farmers, research in small ruminant diseases has been neglected (CARD/GOZ 1993).

MAP1-B is a partial recombinant protein antigen containing amino acids 47–92 of the mature major

antigenic protein 1 (MAP1) of *E. ruminantium* (Van Vliet, Van der Zeijst, Camus, Mahan, Martinez & Jongejan 1995). This protein was found to have higher specificity for *E. ruminantium* when compared to other serologically tested antigens but is recognised by antibodies to *Ehrlichia canis* and *Ehrlichia chaffeensis*, the species affecting dogs and humans, respectively. This study presents the results of a comprehensive serological survey of antibodies reactive to MAP1-B in goats in the communal land areas of Zimbabwe.

MATERIALS AND METHODS

Study areas

The serological survey study was carried out in Mudzi, Bikita and Gwanda districts of Zimbabwe. These districts are all situated in the communal land areas located in the drier agro-ecological regions of the country (Natural Regions III, IV and V) (Fig. 1). They are characterized by low annual rainfall (<650 mm), very little pasture and are mostly suitable for semi-extensive farming (Moyo, O'Keefe & Sill 1993; Muir 1994; Rukuni 1994). Communal land areas

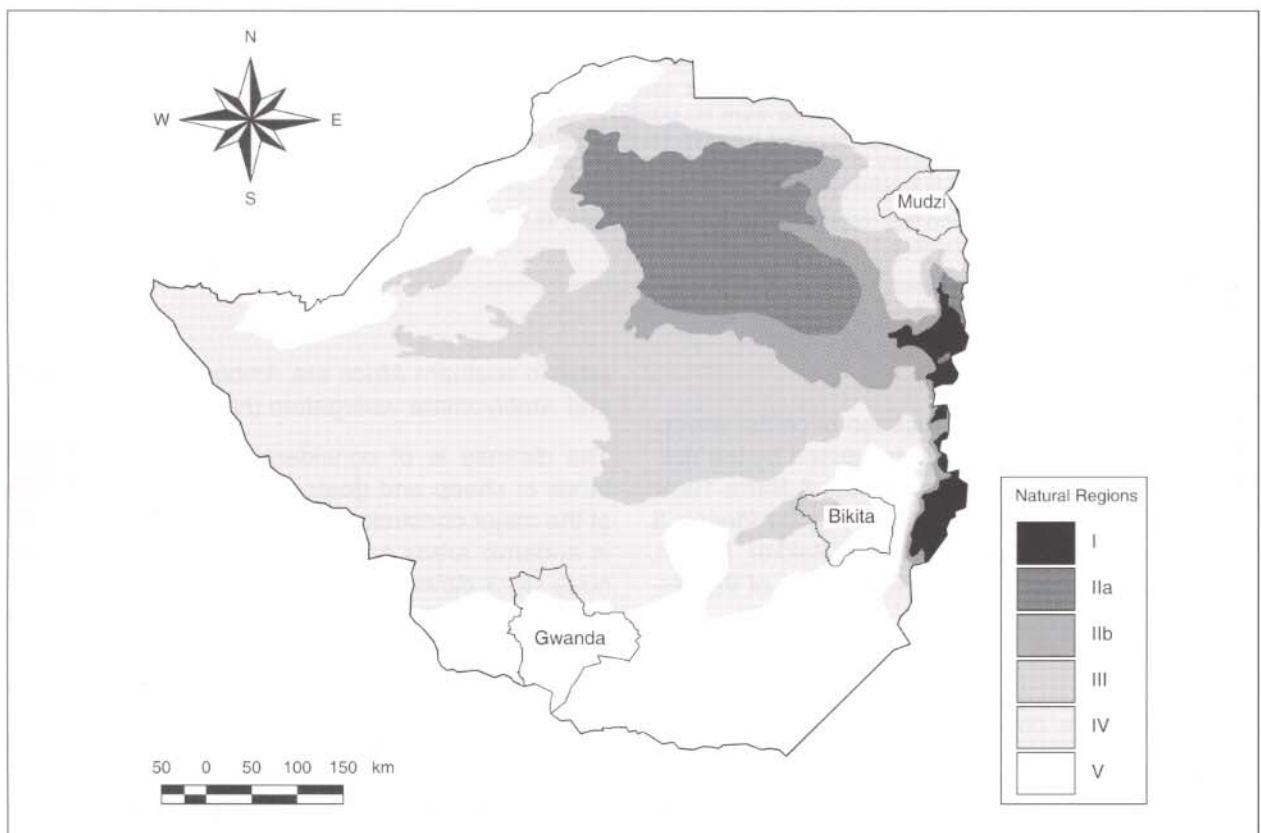


FIG. 1 Map of Zimbabwe showing the agro-ecological natural regions of the country

are characterized by a free-range livestock grazing system whilst small-scale farm livestock is kept under a more intensive livestock management system. The dominant vegetation type is *Colophospermum mopane* (Mopane) woodland. Other common tree species are *Adansonia digitata*, *Kigelia africana* and *Sclerocarya birrhea* (Moyo *et al.* 1993; Muir 1994; Rukuni 1994).

Study design and selection criteria

The study was part of a cross-sectional investigation into the prevalence of haemoparasites among them *E. ruminantium*. A one-stage cluster sampling (Thrusfield 1995) was used due to the difficulty of getting goats to central points. All animals within the cluster, except some kids less than 4 weeks of age because of owner objection, were sampled. The sampling areas were selected randomly from areas with high goat populations in Zimbabwe.

Sample collection

A total of 1 286 goats were sampled in the three districts between March 1999 and February 2000 over three visits. An average of 150 samples were collected from each study area on three occasions during the year. The samples were collected in the rainy season, a period of high tick activity and in the dry season when ticks are present in low numbers. The average number of clusters in Bikita and Mudzi ranged from 15–21, with an average of seven to ten animals per cluster. In Gwanda the average was three clusters with about 50 animals each. Blood was collected in plain tubes for the separation of sera that was kept frozen at -20°C until the time of the test.

Ticks were collected from the animals at the time of serum collection and identified by the Tick unit of the Central Veterinary Laboratories in Harare.

Indirect MAP1-B ELISA methodology

The indirect MAP1-B ELISA was used to screen field samples for antibodies to *E. ruminantium*. The method employed was as described by Van Vliet *et al.* (1995) with a few modifications as described in Semu, Peter, Mukwedeya, Barbet, Jongejan & Mahan (2001). Other modifications included the use of 1% non-fat dry milk (Regilait®, Belgium) for non-specific protein binding and rat anti-goat antibodies (Nordic, The Netherlands) conjugated to horseradish peroxidase was used at a concentration of 0.5g/ml in 0.5 M carbonate buffer, instead of Protifar milk and a rabbit anti-goat conjugate from Kirkegaard

and Perry Labs. The MAP1-B antigen was derived from the Senegal isolate of *E. ruminantium* and was employed at a dilution of 1:600. The test was optimized using these two reagents before the field samples were assayed. Each serum sample was tested in duplicate and the duplicate samples of negative and positive control sera were included in each plate. Optical densities of the completed ELISAs were measured with a Titertek Multiskan ELISA reader (Titertek, Flow Laboratories Inc) using dual wavelengths, 405 and 492 nm. Reactivity was expressed as a percentage, using the optical density of the test sample over the positive control serum. The cut-off point was determined by doubling the negative control reactivity as a percentage of the positive control serum sample (Van Vliet *et al.* 1995; Semu *et al.* 2001).

Data analysis

Prevalence and the differences in prevalence in each of the three sampling areas and three sampling times were calculated using SAS Edition 6.03, General linear model package, 1988 (Model:Prevalence=area.season).

RESULTS

The cut-off points for the 28 ELISA plates averaged at $13.2 \pm 2.8\%$.

The percentage of animals found positive for antibodies to heartwater was 89.4% ($n = 480$) in Mudzi, 83.2% in Bikita ($n = 365$) and 79.4% in Gwanda ($n = 441$), as presented in Fig. 2. The highest prevalence of MAP1-B antibodies in all three areas was during the mid rainy season in the period January-March and the lowest in the drier July-September period. There were no significant differences in the seroprevalence between the three sampling areas and seasons.

The ranges of reactivity of sera of the three areas are presented in Fig. 3. The trend in distribution of reactivity follows a similar pattern for all three areas with a peak in the range 21–60% for Mudzi, Gwanda and Bikita.

All the major genera of ticks found on cattle in Zimbabwe were also found on goats. In Bikita and Gwanda *Rhipicephalus evertsi evertsi* was the predominant tick species, but *A. hebraeum* and *Boophilus decoloratus* were also found. In Mudzi the predominant tick species were *Hyalomma marginatum rufipes* and *Hyalomma truncatum* while *R. evertsi evertsi* occurred in low numbers.

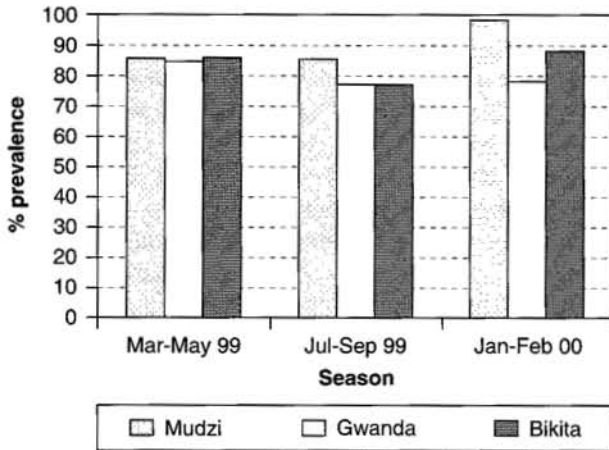


FIG. 2 The seroprevalence of antibodies to the MAP1-B antigen in smallholder sector goats of Zimbabwe

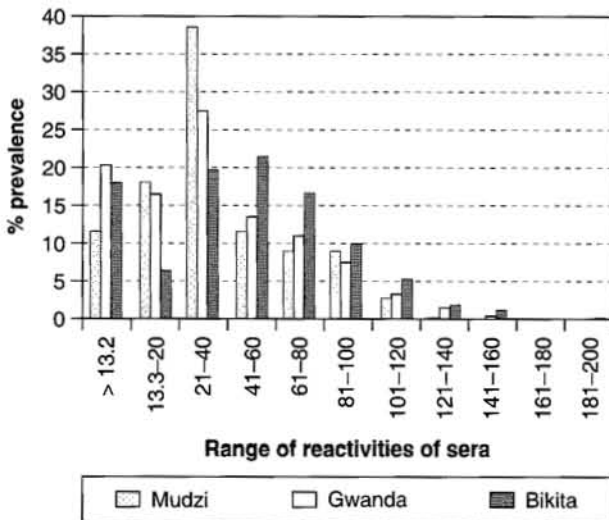


FIG. 3 The range of reactivities of the sera tested from the three study areas. Note that the highest prevalence is in the 21–40 range for Mudzi and Gwanda and in the 41–60 range for Bikita. The < 13.2 range represents the negative sera. The general trend of reactivity is similar for all three areas with the reactivities concentrated in the 21–80 range whilst declining gradually. The sera from Heartwater endemic (Bikita, Gwanda) area does not react differently from that from the non-endemic area (Mudzi)

The prevalence of *E. ruminantium* MAP1-B antibodies was 83.2% (SE ± 5.9) in Bikita, 79.4% (SE ± 3.8) in Gwanda and 89.4% (SE ± 7.5) in Mudzi. The prevalence differences amongst the three study sites were not significant (P value = 0.1995). The prevalence of *E. ruminantium* MAP1-B antibodies was 84.9% (SE ± 0.9) in the late rainy season, 79.3% (SE ± 14.8) in the dry season and 87.9% (SE ± 10.0) in the mid rainy season. The difference

in prevalence between the different seasons was not significant (P value = 0.3205).

DISCUSSION

Bikita and Gwanda are located in the south-western lowveld of Zimbabwe where heartwater and the bont tick, *A. hebraeum*, are endemic. Seroprevalence rates of 83.2% and 79.4% obtained for Bikita and Gwanda respectively, are similar to findings by Mahan, Semu, Peter & Jongejan (1998) and Peter, O'Callaghan, Medley, Perry, Semu & Mahan (2001). They reported a prevalence of *E. ruminantium* antibodies in goats of about 90% in heartwater-endemic areas. The high seroprevalence in Mudzi (89.4%) is unexpected as neither *A. hebraeum* nor *A. variegatum* have been found in this area (Norval 1983; Peter, Perry, O'Callaghan, Medley, Shumba, Madzima, Burrige & Mahan 1998a), although Peter, Perry, O'Callaghan, Medley, Shumba, Madzima, Burrige & Mahan (1998b) have shown that *A. hebraeum* has gradually spread north from its traditional focus in the south of the country. However, a lower prevalence would have been expected in Mudzi area compared to the other two areas where the vectors and disease have been established for over a 100 years. There is a possibility that the animals could be contracting heartwater from Mozambique as a physical barrier between the two countries does not exist in this area. Asselbergs *et al.* (1993) have reported *A. variegatum* in the Tete Province of Mozambique which borders on the Mudzi district. Animals are reported to graze across the border for several days before returning home. However, no *Amblyomma* ticks were observed on animals (including cattle) in the area during the sampling period. In addition it is possible that the animals were purchased from an endemic area and brought into this region and because there are no vectors, heartwater has not been reported.

If heartwater exposure is not the cause of the high seroprevalence in Mudzi, this could be due to the presence of an agent that serologically cross reacts with *E. ruminantium*. The MAP1-B ELISA was validated using sera of all known organisms that were noted to be serologically related to *E. ruminantium* (Jongejan, Wassink, Thielemans, Perie & Uilenberg 1989; Du Plessis 1993; Jongejan, De Vries, Nieuwenhuijs, Van Vliet & Wassink 1993; Mahan *et al.* 1993; Martinez 1993; Van Vliet *et al.* 1995; Matthewman, Kelly, Mahan, Semu, Mason, Bruce, Brouqui & Raoult 1994). Although the MAP1-B ELISA was

found to have a higher specificity for *E. ruminantium* when compared to other serological tests, the obvious shortcoming of this test is that exposure to any unknown *Ehrlichia* or antigenically related organism will induce antibodies that react with the MAP1-B protein. Van Vliet *et al.* (1995) demonstrated that the MAP1-B antigen was recognised by sera to *E. chaffeensis* and *E. canis*. It has also been reported that apathogenic *E. ruminantium* like the Vosloo isolate in South Africa (Du Plessis 1993) and the *Ehrlichia*-like Omatjenne agent in Namibia (Allsopp, Visser, Du Plessis, Vogel & Allsopp 1997) can be the cause of positive reactions in heartwater-free areas. In South Africa, 42% of Boer goats from a heartwater-free and *Amblyomma* tick-free area also gave reactions for the apparently apathogenic *Ehrlichia*-like organism (Omatjenne) (Allsopp *et al.* 1997). The "Omatjenne organism" was first isolated by infecting a mouse with a homogenate of a *Hyalomma truncatum* tick originating from a non-heartwater and non-*Amblyomma* area of Namibia where cattle had tested seropositive for *Ehrlichia* on IFAT. Both organisms are suspected to be transmitted by ticks of the genera *Hyalomma* and *Rhipicephalus* (Du Plessis 1993; Allsopp *et al.* 1997). *E. canis* and *E. chaffeensis* share antigenic determinants with the MAP1-B antigen. It is possible that the former parasite is present in Mudzi and is causing the cross-reactions noticed. Alternatively a new cross-reacting agent may be responsible for the high seroprevalence. The only way to determine this is by isolating and characterizing the agent that elicits that response.

On analysis of the data, there were no significant differences in the prevalence of antibodies with each collection period and between the sampling areas. The highest prevalence figures were recorded in the wet January to February collection period (peak tick activity) and the lowest in the dry July to September period (minimal tick activity) (Norval 1994). The fact that the highest seroprevalence coincides with the peak tick activity suggests that the agent responsible for the cross-reactivity is most likely tick-transmitted. The tick species found on goats in Mudzi were the red-legged tick, *R. evertsi evertsi* and the bont-legged ticks, *H. truncatum* and *H. marginatum rufipes*. Savadye, Kelly & Mahan (1998) reported positive reactions by immunoblotting against *E. ruminantium* antigen on seven heartwater-free farms in Zimbabwe ranging from 8–94%. They attributed these results to an association with the presence of *R. evertsi evertsi*, *H. marginatum rufipes* and *H. truncatum* (Savadye *et al.* 1998). Tick transmission studies revealed transmission of an

agent that cross-reacted serologically with *E. ruminantium*. The ticks responsible for transmitting the agent were PCR positive for an ehrlichial agent based on the 16S rDNA sequence of ehrlichias (Savadye *et al.* 1998). Although the indirect MAP1-B ELISA is the best tool available for the screening of sera for heartwater antibodies (Van Vliet *et al.* 1995) our results show that the test may be detecting false positives (positives from non-heartwater areas).

Since the prevalence data obtained from the three districts are comparable this casts doubt on the performance of the assay in field samples and the acceptability of results even from the endemic areas. Sera from endemic areas (Bikita and Gwanda) and those from the non-endemic area (Mudzi) could not be distinguished from one another on the basis of reactivity. The diagnostic reliability of a given test may differ when used in different regions and should be determined by testing samples from well-defined negative (non-infected) and positive (infected) animals reflecting the target population (Kramps & Van Rooij 1998). Although the MAP1-B indirect Elisa has a high specificity and sensitivity with known negative and positive serum samples, it is nevertheless important that in the validation of the MAP1-B Elisa in Zimbabwe or any region, a representative sample of the goat (or any other target) population is tested. Further evaluations of field sera from endemic and non-endemic areas by the MAP1-B ELISA as well as studies by alternative assays may be required in order to fully evaluate the diagnostic potential of the test (Mahan *et al.* 1998). Peter *et al.* (2001) determined that cattle in heartwater endemic areas of Zimbabwe show a low sero-prevalence compared to the expected high reactivity. In these areas, heartwater is endemic and tick control is minimal, but the sero-prevalence of cattle ranges between 20–60% that was dependent on the region sampled. The authors concluded that the indirect MAP1-B Elisa was not a reliable test to detect exposure to *E. ruminantium*. In a controlled study Semu *et al.* (2001) discovered that the low seroprevalence in cattle sera was as a result of down-regulation of antibody responses to the MAP1-B antigen following recovery from primary clinical infection with *E. ruminantium*. These studies demonstrated that serology for heartwater is an unreliable indicator of past exposure to *E. ruminantium*, and more specific and sensitive assays such as the PCR assay need to be applied in order to detect the organism itself and not exposure to infection. Semu *et al.* 2001 demonstrated that it was possible to detect *E. ruminantium* in the MAP1-B negative cat-

tle by PCR and xenodiagnosis, though sporadically. This study has endorsed the finding that there is a need to develop a serological test that is specific to *E. ruminantium* and excludes any cross-reactions.

There is also a need to redefine heartwater and non-heartwater endemic areas in Zimbabwe on a regular basis. Though no *Amblyomma* ticks were found on domestic ruminants of Mudzi during the study, the positive samples from the area can only be classified as false positives by regular, frequent and properly conducted tick surveys that confirm the absence of these ticks.

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