



## Epidemiology of tick-borne diseases of cattle in Botshabelo and Thaba Nchu in the Free State Province

KARIN DREYER, L.J. FOURIE\* and D.J. KOK

Department of Zoology and Entomology, University of the Orange Free State  
P.O. Box 339, Bloemfontein, 9300 South Africa

### ABSTRACT

DREYER, KARIN, FOURIE, L.J. & KOK, D.J. 1998. Epidemiology of tick-borne diseases of cattle in Botshabelo and Thaba Nchu in the Free State Province. *Onderstepoort Journal of Veterinary Research*, 65:285–289

A seroepidemiological study was conducted on 151 cattle from the Botshabelo and Thaba Nchu areas in the central Free State Province of South Africa, two areas where small scale, peri-urban cattle farming is practised. An indirect fluorescent antibody test was used to test for *Babesia bigemina* and *B. bovis* antibodies. To test for *Anaplasma marginale* antibodies a competitive inhibition enzyme-linked immunosorbent assay method was used. There were no significant differences in serological test results between the cattle from Botshabelo and those from Thaba Nchu. The herd (two areas combined) had an average seroprevalence of 62,42% to *B. bigemina*, 19,47% to *B. bovis* and 98,60% to *A. marginale*. Based on the percentage of cattle that were seropositive to *B. bigemina* the immune status of cattle in the Botshabelo-Thaba Nchu area is approaching a situation of endemic stability. With reference to *A. marginale*, the high seroprevalence is indicative of a situation of endemic stability. The occurrence of *B. bovis* antibodies in the cattle is difficult to explain as *Boophilus microplus* ticks do not occur in the area in which the study was conducted.

**Keywords:** *Anaplasma*, *Babesia*, *Boophilus*, Botshabelo, communal grazing, peri-urban livestock farming, Thaba Nchu, tick-borne disease, ticks

### INTRODUCTION

World-wide, babesiosis and anaplasmosis are considered to be amongst the most important tick-borne diseases of cattle (Morzaria 1986). The economic impact can be expressed in terms of mortality, loss of production, cost of control and, in some cases, restrictions placed on the movement of animals (Norval, Barrett, Perry & Mukhebi 1992).

In South Africa, the known vectors of *Babesia bigemina* are *Boophilus decoloratus*, *Boophilus microplus* and *Rhipicephalus evertsi evertsi*, and for *Babesia bovis*, *Boophilus microplus* (De Vos 1979; De Vos

& Jorgensen 1992). Two species of *Anaplasma*, namely *A. marginale* and *A. centrale*, are known to infect cattle, but *A. centrale* generally produces a mild disease (Potgieter & Stoltsz 1994). In South Africa, five ixodid ticks, namely *Boophilus decoloratus*, *B. microplus*, *Hyalomma marginatum rufipes*, *Rhipicephalus evertsi evertsi* and *R. simus* are the principal biological vectors of *A. marginale* (Potgieter 1979). Various haematophagous insects, including biting fly species of the genus *Tabanus* (Potgieter & Stoltsz 1994) and the stable fly (*Stomoxys calcitrans*), can transmit *A. marginale* mechanically (Potgieter, Sutherland & Biggs 1981).

If tick infestations on cattle are controlled by regular application of acaricides, the source of infection of tick-borne diseases can, to a large extent, be eliminated or considerably reduced. This results in a situation

\* Author to whom correspondence is to be directed

Accepted for publication 29 September 1998—Editor

in which endemic instability to the diseases in question prevails. In Botshabelo and Thaba Nchu, two urban areas situated about 60 km east of Bloemfontein in the central highveld area of the Free State Province of South Africa, most of the farmers who make use of the commonage in and around these towns to graze their stock, either use no tick control methods or old engine-oil is used as an acaricide. High numbers of ticks, predominantly *B. decoloratus*, were recorded during a study on tick infestations of cattle in the areas (Dreyer 1997). Before an integrated tick control management plan can be advocated for a specific area, accessible, accurate data on the occurrence of tick-borne diseases are required. In areas where such data are difficult to obtain owing to no or inaccurate record keeping, such as in Botshabelo and Thaba Nchu, serological studies can provide useful information.

The aim of this investigation was to determine the existing immune status of cattle in Botshabelo and Thaba Nchu to *Babesia bovis*, *B. bigemina* and *A. marginale* infections by conducting a seroepidemiological study.

## MATERIALS AND METHODS

From March to June 1996, a total of 151 cattle were sampled in the Botshabelo and Thaba Nchu areas. In Botshabelo, 115 animals were used and in Thaba Nchu, 36 animals. The cattle were of mixed-breed origin, predominantly Friesian and Brown Swiss crosses and belonged to several owners. The animals used in the study included heifers, cows, bulls and oxen, all between 6 months and 4 years of age. Age determination was based on dentition (West 1988). During the day all the animals grazed as mixed herds on communal pastures known to harbour ticks, and in the evenings they were collected and the lactating cows milked. At night they were kept in kraals in the backyards of their owners (Dreyer 1997).

During the sampling procedure, the animals were restrained in a mobile handling facility consisting of a race, crush and neck clamp. Blood samples were collected aseptically from the jugular or coccygeal veins into sterile serology tubes. The tubes with the whole blood were left overnight at room temperature to allow clotting. Sera were separated by centrifugation in a standard MSE Minor centrifuge at 3 000 rpm for 5–10 min. The sera were stored in 1,8 ml vials (Nunc Cryo Tube Vials, Weil Organization Distributors, Johannesburg), in an ultra-deep freezer at  $-70^{\circ}\text{C}$ .

Serological tests were performed in the Protozoology Section, Onderstepoort Veterinary Institute (OVI). An indirect fluorescent antibody test (IFA-test), described by Morzaria, Brocklesby & Harradine (1977), was used to detect *Babesia bigemina* and *B. bovis* antibodies, and for *A. marginale* antibodies, a competitive inhibition enzyme-linked immunosorbant as-

say (CI-ELISA) method was employed (Visser, McGuire, Palmer, Davis, Shkap, Pipano & Knowles 1992).

Statistical data analyses were done on an IBM-compatible microcomputer. The software application used was SPSS Release, version 4.0 for Convex UNIX. A two-tailed Fisher exact test (Zar 1974), which is a binomial, non-parametric test for proportions, was applied to determine differences in the numbers of seropositive animals between those from Botshabelo and those from Thaba Nchu. A two-tailed, paired Student's *t*-test (Barnard, Gilbert & McGregor 1993) was used to determine significant differences in the geometric mean titre (GMT) of *B. bigemina* and *B. bovis* antibodies in Botshabelo, as compared to Thaba Nchu. According to the method described by Thrusfield (1986), only seropositive animals can be used in calculating the GMT to indicate possible differences in probabilities of recent infections or outbreaks. The arithmetic mean or coded mean was calculated by coding the titres (Table 1), and dividing the sum of the coded titres by the number of titres.

The GMT is the antilog<sub>2</sub> of the coded mean (P). When an initial log<sub>10</sub> dilution has been carried out, subsequently followed by log<sub>2</sub> dilutions, the GMT is calculated as follows:

$$\begin{aligned} \text{GMT}/10 &= 2^x \\ \log_{10}(\text{GMT}/10) &= x \times 0,301 \\ \text{GMT}/10 &= \text{antilog}(x \times 0,301) \\ \text{GMT} &= \text{antilog}(x \times 0,301) \times 10 \end{aligned}$$

A significance level of  $P \leq 0,05$  was used throughout. Data on seropositive animals are presented as percentages of the total samples to simplify the interpretation of results.

## RESULTS

The results were divided into two groups according to the location where serum samples were collected, namely Botshabelo or Thaba Nchu. The results of the three serological testings are summarized in Table 2. Data on seropositive animals are presented as percentages of total samples to simplify the interpretation of results. A comparison between the two locations showed no significant differences in serological test results between the cattle from Botshabelo and those from Thaba Nchu. The 151 cattle had an

TABLE 1 Antibody titres expressed as reciprocal dilutions (X) and coded titres (log<sub>2</sub> X)

Reciprocal dilution (X)	Coded titre (log <sub>2</sub> )
80	1
160	2
320	3

TABLE 2 Prevalence of antibodies against *Babesia bigemina*, *B. bovis* and *Anaplasma marginale* in cattle ( $n = 151$ ) in Botshabelo and Thaba Nchu areas

Area	Sample size	% Positive to <i>Babesia bigemina</i>	% Positive to <i>Babesia bovis</i>	% Positive to <i>Anaplasma marginale</i>
Botshabelo	115	61,99	18,57	98,19
Thaba Nchu	36	65,31	22,87	100,00
Combined	151	62,42	19,47	98,60

TABLE 3 The geometric mean titres (GMT) of *Babesia bigemina* and *B. bovis* in cattle in the Botshabelo-Thaba Nchu area

Haemoparasites	Botshabelo GMT	Thaba Nchu GMT	Significance ( $P < 0,05$ )
<i>Babesia bigemina</i>	45,6	40,0	NS
<i>B. bovis</i>	24,9	30,8	NS

average seroprevalence of 62,42% to *B. bigemina*, 19,47% to *B. bovis* and 98,60% to *A. marginale*.

Geometric mean titres (GMTs) of antibodies against *B. bigemina* and *B. bovis* were determined for both the Botshabelo and Thaba Nchu areas. There were no significant differences in the GMTs of the cattle from Botshabelo and those from Thaba Nchu (Table 3).

## DISCUSSION

Based on the information gained from a serological study, the immune status of cattle in an area can be classified into an endemically stable (81–100% positive sera) situation, a situation approaching stability (61–80%), an unstable (21–60%) situation, a minimal disease situation (1–20%), and a disease-free situation (0% positive sera) (Norval, Fivaz, Lawrence & Daillecourt 1983). Where the inoculation rate of *Babesia* or *Anaplasma* is adequate to ensure that all calves are infected while they are protected by innate and/or colostral immunity, clinical disease is minimal and endemic stability is achieved. The disruption of an existing situation of endemic stability is usually associated with drought conditions (De Vos 1979), or increased tick control (Norval *et al.* 1983). Endemic instability, on the other hand, describes the situation in a herd where some animals fail to become infected within nine months of birth. When such susceptible individuals encounter infected ticks, clinical disease develops (Howell, De Vos, Bezuidenhout, Potgieter & Barrowman 1981).

Based on the percentage of cattle that were seropositive (62,42%) to *B. bigemina*, it is evident that cattle in the Botshabelo-Thaba Nchu area fit into the group of infection rates that is indicative of a situa-

tion approaching endemic stability, but with a possible risk of outbreak of disease. No clinical cases of redwater due to this organism were observed during a 12 months clinical health study on cattle in this area (Dreyer 1997). Although there were differences in seroprevalence between the cattle in the Botshabelo and Thaba Nchu areas, these differences were small and non-significant.

The average seroprevalence of 19,47% against *B. bovis* for cattle sampled in the Botshabelo-Thaba Nchu area indicated a situation of minimal disease (1–20%), and the risk of clinical disease outbreak due to this organism is thus small. As far as is known, *B. bovis* is only transmitted by *Boophilus microplus* (De Vos 1979; De Vos & Jorgensen 1992) which does not occur in the Free State Province (Howell, Walker & Nevill 1983).

In a study on tick infestations conducted over a 12 month period in the Botshabelo-Thaba Nchu region, high numbers of *B. decoloratus* were collected and identified, but no *B. microplus* ticks were found (Dreyer 1997). The occurrence of *Babesia bovis* antibodies in the cattle is difficult to explain.

It is possible that the IFA-test used to detect antibodies against *B. bovis* was influenced by the high levels of those against *B. bigemina*. Leeflang & Perie (1972) and Gray & De Vos (1981) observed serological cross-reactions between the two species and false positive *B. bovis* reactions were seen with the IFA-tests they used. In a study done by Bessenger & Schoeman (1983) sera containing antibodies against *B. bigemina* reacted positively with a *B. bovis* antigen in a IFA-test, although the titres obtained were consistently lower than those obtained with *B. bovis* sera.

Although several studies concluded that *Boophilus microplus* is the sole transmitter of *Babesia bovis* in southern Africa (De Vos 1979; De Vos & Every 1981; Howell *et al.* 1981; Norval *et al.* 1983), it is possible that other ticks can potentially transmit *B. bovis* in the area. Studies in Nigeria (Ilemobade 1992) have indicated a more widespread distribution of *B. bovis* than the scanty distribution of the vectors *Boophilus annulatus* and *Boophilus geigy* in that country, suggesting that the identification of the vectors of *B. bovis* in Nigeria needs further investigation. Ilemobade (1992)

has speculated that *Boophilus decoloratus* might be the vector of *B. bovis* in some parts of Nigeria.

Another possibility for the relatively high seroprevalence of antibodies against *B. bovis*, that were obtained in this study, is that the organism could have been mechanically transmitted by haematophagous insects although this seems improbable and the occurrence of *B. bovis* antibodies in cattle in the Thaba Nchu-Botshabelo area needs further investigation. It is interesting to note that the State Veterinarian of the region who is stationed in Thaba Nchu, has not diagnosed any *B. bovis* infections in the last six years of record keeping (W. Stöhr, personal communication 1995).

When comparing coded antibody titres in populations, two parameters must be considered before inferences are made: the relative proportion (percentage) of seropositive animals, irrespective of titres, and the GMTs of the seropositive populations.

In the present study, it was established that there were no significant differences between GMTs of both *B. bigemina* and *B. bovis* in the Botshabelo and Thaba Nchu areas, respectively. A high GMT is indicative of a recent epidemic and a low GMT, merely of the persistence of antibodies in convalescent animals (Thrusfield 1986). The GMTs to *B. bigemina* and *B. bovis*, in the Botshabelo and Thaba Nchu areas respectively, were low, which indicate a persistence of resistance in the animals in the absence of recent extensive outbreaks.

The average seroprevalence for *A. marginale* was 98.6%. A seroprevalence above 81% is indicative of a situation of endemic stability, but clinical cases of anaplasmosis were diagnosed in the areas concerned during a recent 12 month study on herd health (Dreyer 1997). In an extensive country-wide study conducted in Zimbabwe, the absence of a relationship between the prevalence of *Anaplasma* antibodies and clinical cases of anaplasmosis in cattle could not be explained (Norval, Fivaz, Lawrence & Brown 1984). Host immunity to any disease including anaplasmosis requires a healthy immune system. It is possible that the long lactations of at least 9 months, a high tick challenge, the harsh winters and no or insufficient supplementary feeding had an influence on the generally poor physical condition of the animals (Dreyer 1997), resulting in a decline in immunocompetence of some of the cattle with the appearance of clinical anaplasmosis.

The results of a study conducted in various parts of South Africa (De Vos & Potgieter 1983) indicated that the highest percentage of bovine babesiosis outbreaks occurred on farms on which moderate tick control measures were applied. These led to a situation of endemic instability with infection rates of 20–70%. The present serological study has provided a

quantitative assessment of antibody prevalence rates against the babesiosis and *A. marginale* infections present in the area, and can be used as a basis for the planning of tick control strategies and the control of tick-borne diseases in the area.

In the Botshabelo-Thaba Nchu area no regular measures to control ticks were practised in the past which has resulted in the challenge of calves by certain tick-borne pathogens, and the development of a situation of endemic stability against *A. marginale* and one approaching endemic stability against *B. bigemina*. An intensive tick control plan in the area can therefore not be justified, but an integrated tick management plan should be introduced in order to control the deleterious effects of the ticks on their hosts which in some cases appear to be severe.

## ACKNOWLEDGEMENTS

This study was funded by the Foundation for Research Development and the University of the Free State. The technical assistance of Mr Eddie Williams and the assistance of Dr J.M. van Zyl in the statistical analyses of the data are greatly appreciated.

## REFERENCES

- BARNARD, C.J., GILBERT, F.S. & MCGREGOR, P.K. 1993. *Asking questions in biology*. Harlow: Longman Scientific & Technical.
- BESSENGER, R. & SCHOEMAN, J.H. 1983. Serological response of cattle to infection with *Babesia bigemina* and *Babesia bovis* in Southern Africa. *Onderstepoort Journal of Veterinary Research*, 50:115–117.
- DE VOS, A.J. 1979. Epidemiology and control of bovine babesiosis in South Africa. *Journal of the South African Veterinary Association*, 50:357–362.
- DE VOS, A.J. & EVERY, R. 1981. Epidemiology and control of bovine babesiosis in the Natal Midlands, in *Tick biology and control: Proceedings of an International Conference held at the Tick Research Unit, Rhodes University, Grahamstown, South Africa, 27–29 January, 1981*, edited by G.B. Whitehead & J.D. Gibson. Grahamstown: Tick Research Unit, Rhodes University.
- DE VOS, A.J. & JORGENSEN, W.K. 1992. Protection of cattle against babesiosis in tropical and subtropical countries with a live, frozen vaccine, in *Tick vector biology: Medical and veterinary aspects*, edited by B. Fivaz, T. Petney & I.G. Horak. Berlin: Springer-Verlag.
- DE VOS, A.J. & POTGIETER, F.T. 1983. The effect of tick control on the epidemiology of bovine babesiosis. *Onderstepoort Journal of Veterinary Research*, 50:3–5.
- DREYER, KARIN. 1997. Occurrence and control of parasites on cattle in urban and peri-urban environments with specific reference to ticks. M.Sc. thesis, Bloemfontein: University of the Free State.
- GRAY, J.S. & DE VOS, A.J. 1981. Studies on a bovine *Babesia* transmitted by *Hyalomma marginatum rufipes* Koch, 1844. *Onderstepoort Journal of Veterinary Research*, 48:215–223.
- HOWELL, C.J., DE VOS, A.J., BEZUIDENHOUT, J.D., POTGIETER, F.T. & BARROWMAN, P.R. 1981. The role of chemical tick eradication in the control or prevention of tick-transmitted diseases of cattle, in *Tick Biology and Control: Proceedings*

- of an International Conference held at the Tick Research Unit, Rhodes University, Grahamstown, South Africa, 27–29 January, 1981, edited by G.B. Whitehead & J.D. Gibson. Grahamstown: Tick Research Unit, Rhodes University.
- HOWELL, C.J., WALKER, J.B. & NEVILL, E.M. 1983. *Ticks, mites and insects infesting domestic animals in South Africa. Part 1: Descriptions and biology*. Pretoria: Department of Agricultural Technical Services (Science Bulletin, no. 393).
- ILEMOBADE, A.A. 1992. Anaplasmosis, babesiosis and cowdriosis in the West African sub-region: distribution, economic importance and control measures, in *Recent Developments in the Control of Anaplasmosis, Babesiosis and Cowdriosis: Proceedings of a Workshop Held at ILRAD, Nairobi, Kenya, 13–15 May 1991*, edited by T.T. Dolan. Nairobi: The International Laboratory for Research on Animal Diseases.
- LEEFLANG, P. & PERIÈN, M. 1972. Comparative immunofluorescent studies on 4 *Babesia* species of cattle. *Research in Veterinary Science*, 13:342–346.
- MORZARIA, S.P. 1986. Cattleba besiosis. *Kenya Veterinarian*, 10:8–9.
- MORZARIA, S.P., BROCKLESBY, D.W. & HARRADINE, D.L. 1977. Evaluation of the indirect fluorescent antibody test for *Babesia major* and *Theileria mutans* in Britain. *The Veterinary Record*, 100:484–487.
- NORVAL, R.A.I., BARRETT, J.C., PERRY, B.D. & MUKHEBI, A.W. 1992. Economics, epidemiology and ecology: a multi-disciplinary approach to the planning and appraisal of tick and tick-borne disease control in Southern Africa, in *Tick vector biology: Medical and veterinary aspects*, edited by B. Fivaz, T. Petney & I.G. Horak. Berlin: Springer-Verlag.
- NORVAL, R.A.I., FIVAZ, B.H., LAWRENCE, J.A. & BROWN, A.F. 1984. Epidemiology of tick-borne diseases of cattle in Zimbabwe. II: Anaplasmosis. *Tropical Animal Health and Production*, 16:63–70.
- NORVAL, R.A.I., FIVAZ, B.H., LAWRENCE, J.A. & DAILLECOURT, T. 1983. Epidemiology of tick-borne diseases of cattle in Zimbabwe. I. Babesiosis. *Tropical Animal Health and Production*, 15:87–94.
- POTGIETER, F.T. 1979. Epizootiology and control of anaplasmosis in South Africa. *Journal of South African Veterinary Association*, 50:367–372.
- POTGIETER, F.T. & STOLTZ W.H. 1994. Bovine Anaplasmosis, in *Infectious diseases of livestock with special reference to Southern Africa, 1*, edited by J.A.W. Coetzer, G.R. Thomson, R.C. Tustin & N.P.J. Kriek. Cape Town: Oxford University Press.
- POTGIETER, F.T., SUTHERLAND, B. & BIGGS, H.C. 1981. Attempts to transmit *Anaplasma marginale* with *Hippobosca rufipes* and *Stomoxys calcitrans*. *Onderstepoort Journal of Veterinary Research*, 48:119–122.
- THRUSFIELD, M. 1986. *Veterinary Epidemiology*. London: Butterworth & Co. Ltd.
- VISSER, E.S., MCGUIRE, T.C., PALMER, G.H., DAVIS, W.C., SHKAP, V., PIPANOE, & KNOWLES JR, D.P. 1992. The *Anaplasma marginale* msp5 gene encodes a 19 kilodalton protein conserved in all recognized *Anaplasma* species. *Infection and Immunity*, 60:5139–5144.
- WEST, G.P. (Ed.) 1988. *Black's veterinary dictionary*, 16<sup>th</sup> ed. London: A & C Black Ltd.
- ZAR, J.H. 1974. *Biostatistical analysis*. Englewood Cliffs, New Jersey: Prentice-Hall, Inc.