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# *Cowdria ruminantium* antibodies in acaricide-treated and untreated cattle exposed to *Amblyomma variegatum* ticks in The Gambia

R.C. MATTIOLI[1,*], M. BAH[1], R. REIBEL[2] and F. JONGEJAN[3,4]
[1]*International Trypanotolerance Centre, Banjul, The Gambia*
[2]*Institute for Parasitology and Tropical Veterinary Medicine, Faculty of Veterinary Medicine, Free University of Berlin, Berlin, Germany*
[3]*Department of Parasitology and Tropical Veterinary Medicine, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands*
[4]*Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa*

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**Abstract.** An indirect enzyme-linked immunosorbent assay (ELISA), based on the major antigenic protein 1 fragment B (MAP1-B) of *Cowdria ruminantium*, was used to assess seroprevalence in cattle in The Gambia. Two groups of 20 N'Dama and 20 Gobra zebu cattle were monitored for 12 months with flumethrin treatment and for another 10 months without acaricidal treatment. Two groups of 20 N'Dama and 20 Gobra cattle served as untreated controls. During the period of acaricidal treatment, the cumulative proportions of positive serum samples were $25.6 \pm 5.6\%$ ($\pm$ confidence interval) and $34.7 \pm 6.8\%$ in treated N'Dama and Gobra cattle respectively; the proportion of positive sera in untreated cattle was $52.2 \pm 6.9\%$ in N'Damas and $61.4 \pm 7.3\%$ in Gobras. Within breed, difference in antibody prevalence between treated and untreated cattle was significant ($P < 0.001$) but between breed differences were not significant. In the 10 months following suspension of acaricide application, there was an increase of proportion of positive serum samples in previously treated N'Dama and Gobra cattle. In both previously treated and untreated animals the peak of positive seroreactions occurred during and subsequent to the period of activity of *Amblyomma variegatum* adults. Cumulative seroprevalences in previously treated N'Dama and Gobra cattle were $32.6 \pm 6.9\%$ and $44.7 \pm 8.5\%$, respectively; in untreated animals seroprevalence was $38.6 \pm 7.2\%$ in N'Dama and $65.3 \pm 8.4\%$ in Gobra cattle. Throughout the study period, within the N'Dama breed, the seropositive rate in previously treated cattle did not differ from that in untreated animals. Conversely, within the Gobra breed, the number of positive seroreactions was higher ($P < 0.002$) in untreated animals than in previously treated cattle. These results provide a support for designing *A. variegatum* and heartwater control strategies, if necessary, in The Gambia in relation to cattle breeds.

**Key words:** *Amblyomma variegatum, Cowdria ruminantium*, Gobra zebu cattle, MAP1-B ELISA, N'Dama, The Gambia

* Author for correspondence: Tel.: +220 46 29 28 / 46 32 70; Fax: +220 46 29 24; E-mail: raf.mattioli@commit.gm

## Introduction

Heartwater (cowdriosis) is a rickettsial infection caused by *Cowdria ruminantium*, affecting wild and domestic ruminants and is transmitted by ticks of the genus *Amblyomma*. Cowdriosis occurs throughout most of sub-Saharan Africa and on several Caribbean islands and it is considered one of the major economically important tick-borne diseases (Provost and Bezuidenhout, 1987).

In West Africa, *A. variegatum* is the main tick species infesting cattle (Koney *et al.*, 1994; Konstantinov *et al.*, 1990), and is the major vector of *C. ruminantium* (Gueye *et al.*, 1993). In Senegal, high mortality due to heartwater was observed in indigenous small ruminants (Gueye *et al.*, 1984). In bovines, lethal cases of heartwater were mainly reported in exotic Red Sindhi $\times$ Sahiwal crossbred and pure Montbeliard cattle (Gueye *et al.*, 1982), while a situation of endemic stability appears to be present in indigenous cattle living in *A. variegatum* infested areas (Gueye *et al.*, 1993). In The Gambia, mortality due to cowdriosis was recently reported in Gobra zebu cattle undergoing an experimental trypanosome infection and exposed to natural tick challenge, while no deaths were observed in trypanosome infected N'Dama or uninfected N'Dama and Gobra zebu cattle (Mattioli *et al.*, 1994). Additionally, in indigenous Djallonke sheep and West African Dwarf goats, reared at the International Trypanotolerance Centre (ITC), a substantial proportion of routinely examined Giemsa-stained post-mortem brain smears was positive for *C. ruminantium*, despite regular acaricide treatments (Mattioli and Jaitner, personal observation, 1998).

Intensive and prolonged tick control is likely to affect endemic stability to tick-borne diseases which generally establishes in indigenous cattle following continuous contact with tick-borne micro-organisms (Du Plessis *et al.*, 1992; Norval *et al.*, 1995). With the exception of recent data derived from a study on a small number of cattle monitored for a short period (Mattioli *et al.*, 1994), no further information is available on heartwater in The Gambia. The objective of the present study was, therefore, to investigate the prevalence of *C. ruminantium* antibodies in intensively acaricide-treated and untreated N'Dama and Gobra zebu cattle exposed for approximately two years to natural *A. variegatum* infestation. The effect of suspension of intensive tick control on the occurrence of *C. ruminantium* antibodies was also explored. The results provide basic information on *A. variegatum* and *C. ruminantium* management programmes aimed at developing a sustainable cattle industry in The Gambia.

## Material and Methods

### *Study sites*

The survey was conducted at the ITC Keneba station (16°01′W; 13°20′N), situated approximately 80 km from the Atlantic coast. The study site is located in the Sudano-Sahelian ecological zone traditionally used for animal production. Rainfall is unimodally distributed from July to October. In this zone the dominant vegetation is open savannah woodland. A more detailed description of the vegetation cover and climatic conditions is provided by Mattioli *et al.* (1998).

### *Serum samples*

A herd composed of N'Dama (20 males and 20 females) and Gobra zebu (20 males and 20 females) cattle, aged 16–20 months at the start of the investigation, was used in the study. The N'Dama cattle were born at the ITC Keneba station. The Gobra zebus were acquired in October 1994 from an area of 50 km to the north. This area is similar to the study site with respect to ecoclimate and tick fauna (Gueye *et al.*, 1989). Both N'Dama and Gobra cattle did not receive regular acaricide treatments prior to the start of the study. Serum samples were collected monthly from February 1995 to December 1996 and stored at −20°C until tested. In this herd, all animals were sprayed twice with flumethrin (0.5% solution) at weekly interval (6 and 13 January 1995), thereafter half of the animals of each breed received monthly acaricide treatments from 27 January 1995 to March 1995, then fortnightly until June 1995. Flumethrin pour-on (1 mg of active ingredient per kilogram body weight) was applied weekly from July 1995 to 7 February 1996. The remaining cattle were left untreated (Mattioli *et al.*, 1998). In the experimental herd, deaths attributed to a nonspecific pneumopathy and excluding heartwater occurring prior to the start of the study and during the period of serum collection progressively reduced number of available sera (Mattioli *et al.*, 1998). On some occasions, serum samples were not available.

### *Serological assay*

Serum samples were tested using an indirect enzyme-linked immunosorbent assay (ELISA), as described by van Vliet *et al.* (1995) and Mboloi *et al.* (1999). The ELISA is based on a specific portion of the major antigenic protein 1 fragment B (MAP1-B) of *C. ruminantium* (van Vliet *et al.*, 1995, 1996). The test was conducted in duplicate for each serum sample. Two negative and two positive control sera, obtained from calves experimentally infected with *C. ruminantium* at the Faculty of Veterinary Medicine, Utrecht, The Netherlands, were also included in duplicate in each plate. When the mean reactivity

of a screened serum sample, expressed by its optical density (OD) measured with an ELISA reader, was equal to or greater than twice that yielded by the negative controls, the sample was considered as positive (van Vliet *et al.*, 1995; Mboloi *et al.*, 1999).

*Tick data*

Data on seasonal dynamics and relative abundance of *A. variegatum* adults are derived from recent investigations conducted on the same animals used in this study (Mattioli *et al.*, 1998). They are supplemented with further unpublished data collected from these cattle by the ITC Animal Health Research Unit on the occurrence of nymphal instars of *A. variegatum*. Briefly, occurring ticks were collected monthly on the right side of animal's body and the whole tail from February to May 1995, fortnightly from June until October 1995 and monthly thereafter. Tick collection was performed both from acaricide-treated and untreated animals immediately prior to flumethrin application. *A. variegatum* numbers reported are the number of ticks of the tail plus twice the number collected on the right side of the body.

*Statistical analysis*

Prevalences of *C. ruminantium* antibody are expressed in percentages, including confidence intervals at 95%. The differences in the cumulative proportions of positive serum samples, between treated and untreated cattle, during and after the periods of acaricidal application were subjected to the $\chi^2$ test. The same statistical approach was applied for the periods of activity of *A. variegatum* nymphs and adults, as assessed in untreated animals. Two levels of comparison were performed: between breeds of cattle belonging to the same group (between-breed comparison) and between groups of animals of the same breed (within-breed comparison).

## Results

Monthly prevalence of *C. ruminantium* antibodies and mean burdens of *A. variegatum* nymphs and adults in acaricide-treated N'Dama and Gobra zebu cattle during and subsequent to the period of acaricide application and in corresponding untreated animals are presented in Figures 1 and 2, respectively.

*Period of acaricide application (February 1995 to February 1996)*

The cumulative prevalence of positive serum samples to *C. ruminantium* MAP1-B antigen was lower in both acaricide-treated N'Dama ($25.6 \pm 5.6\%$)

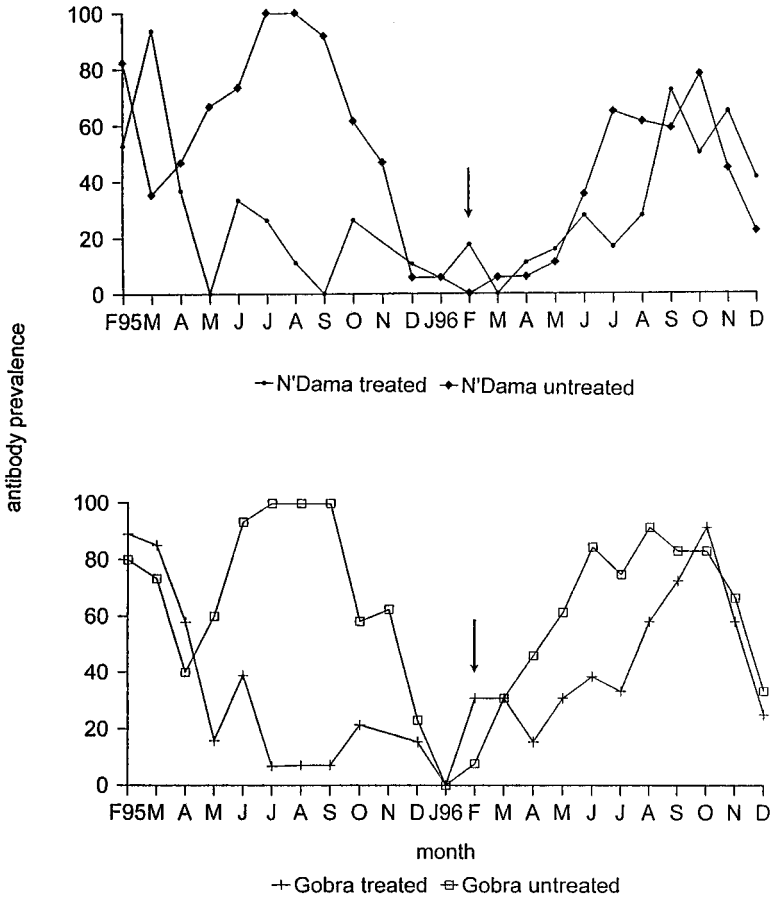


*Figure 1.* Monthly serological prevalence (%) of *C. ruminantium* antibodies, as determined by MAP1-B ELISA test, in acaricide-treated and untreated N'Dama and Gobra zebu cattle (arrow indicates suspension of acaricide treatment; last treatment 7 February 1996).

and Gobra zebu ($34.7 \pm 6.8\%$) compared with that of corresponding untreated cattle ($52.2 \pm 6.9\%$ in N'Dama and $61.4 \pm 7.3\%$ in Gobra cattle) (N'Dama treated vs untreated, $P < 0.001$; Gobra treated vs untreated, $P < 0.001$). Conversely, the proportion of positive serum samples was similar in acaricide-treated N'Dama and Gobra zebu and also in untreated cattle of both breeds (Table 2). During the periods of *A. variegatum* nymphal activity, i.e. February to May 1995, October 1995 to February 1996, as assessed by mean nymph burdens in untreated cattle (mean nymph burdens in untreated animals were 23.5 and 8.8 in Gobra and N'Dama, respectively, and 3.2 in acaricide-treated Gobra and 3.3 in corresponding N'Dama cattle) (Figure 2), no significant within-breed or between-breed differences were detected in

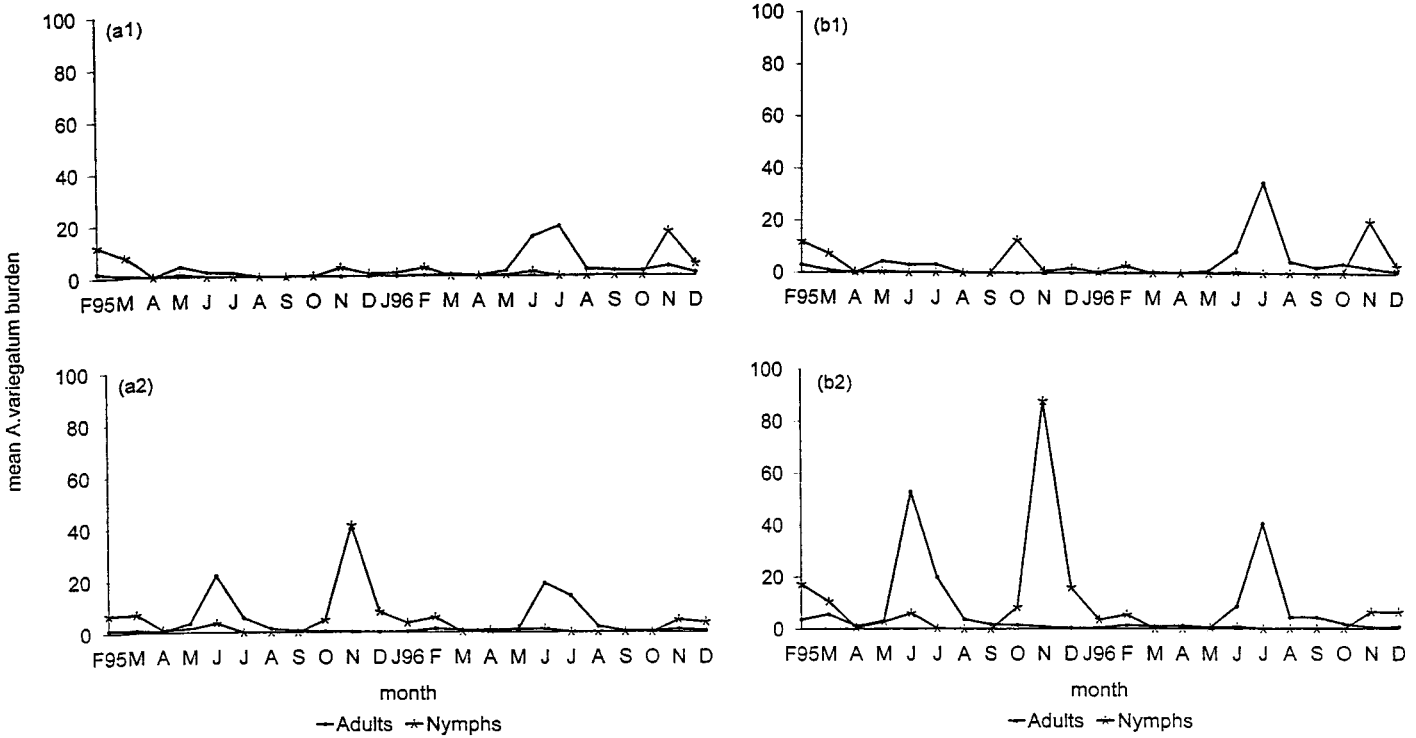


*Figure 2.* Monthly mean burdens of adults and nymphs of *A. variegatum* in acaricide-treated (a1 = N'Dama; b1 = Gobra) (last treatment 7 February 1996) and untreated (a2 = N'Dama; b2 = Gobra) cattle.

*Table 1.* Monthly and total numbers of sera screened for *C. ruminantium* antibodies according to animal breed and acaricide status

| Cattle breed | Acaricide status | Monthly number of serum samples tested | | | | | | | | | | | | | | | | | | | | | | Total sampled |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | F 95 | M | A | M | J | J | A | S | O | N | D | J 96 | F | M | A | M | J | J | A | S | O | N | D | |
| N'Dama | Treated* | 17 | 16 | 19 | 15 | 18 | 19 | 18 | 19 | 19 | – | 19 | 19 | 17 | 17 | 18 | 19 | 18 | 18 | 18 | 18 | 18 | 17 | 17 | 393 |
| N'Dama | Untreated | 17 | 17 | 15 | 15 | 15 | 15 | 15 | 12 | 13 | 15 | 18 | 18 | 18 | 18 | 17 | 18 | 17 | 17 | 18 | 17 | 18 | 18 | 18 | 379 |
| Gobra | Treated* | 18 | 20 | 19 | 19 | 18 | 15 | 14 | 14 | 14 | – | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 12 | 12 | 11 | 12 | 12 | 12 | 313 |
| Gobra | Untreated | 15 | 15 | 15 | 15 | 15 | 13 | 12 | 12 | 12 | 8 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 12 | 12 | 12 | 12 | 12 | 12 | 295 |

*Last treatment 7 February 1996.

seroprevalence of *C. ruminantium* antibodies. On the other hand, between June and September 1995, corresponding to and immediately following the peak activity of *A. variegatum* adults (mean adult burdens were 25.4 and 7.4 in untreated Gobra and N'Dama cattle, respectively, and 1.6 in acaricide-treated Gobras and 0.8 in corresponding N'Damas) seroprevalence of *C. ruminantium* antibodies was lower in acaricide-treated cattle of both breeds ($17.6 \pm 8.7\%$ in N'Damas, $16.4 \pm 9.3\%$ in Gobras) compared with the respective untreated cattle ($91.2 \pm 3.7\%$ and $98.1 \pm 3.7\%$ in N'Dama and Gobra cattle, respectively) (N'Dama treated vs N'Dama untreated, $P < 0.001$; Gobra treated vs Gobra untreated, $P < 0.001$). For the same period, comparison within treatment groups revealed no significant difference in the proportion of positive sera between cattle of the two breeds (Table 2).

*Table 2.* Comparison of serological prevalence of *C. ruminantium* between breeds and within treatment, both during the period of acaricide application (February 1995 to February 1996) and following suspension of acaricidal treatments (March 1996 to December 1996) (periods are sub-divided to reflect *A. variegatum* (Av) adult and nymph seasonal activity (for *A. variegatum* burden corresponding to breed and treatment group *vide* Figures a1, a2, b1 and b2))

| | *C. ruminantium* antibody prevalence (%) | | | |
|---|---|---|---|---|
| Considered period | N'Dama treated | N'Dama untreated | Gobra treated | Gobra untreated |
| Feb. 1995 – May | $46.3 \pm 11.9$ | $57.8 \pm 12.1$ | $61.8 \pm 10.9$ | $71.7 \pm 11.4$ |
| (Av nymph activity) | (31/67*) | (37/64) | (47/76) | (38/60) |
| Jun. – Sep. | $17.6 \pm 8.7a$ | $91.2 \pm 3.7a$ | $16.4 \pm 9.3b$ | $98.1 \pm 3.7b$ |
| (Av adult activity) | (13/74) | (52/57) | (10/61) | (51/52) |
| Oct. 1995 – Feb. 1996 | $14.9 \pm 8.1$ | $20.7 \pm 8.8$ | $17.0 \pm 10.1$ | $30.5 \pm 11.7$ |
| (Av nymph activity) | (11/74) | (17/82) | (9/53) | (16/59) |
| Sub-total | $25.6 \pm 5.6a$ | $52.2 \pm 6.9a$ | $34.7 \pm 6.8b$ | $61.4 \pm 7.3b$ |
| | (55/215) | (106/203) | (66/190) | (105/171) |
| Mar. 1996 – May | $9.3 \pm 7.7$ | $7.5 \pm 7.1a$ | $25.6 \pm 13.7$ | $46.2 \pm 15.6a$ |
| (Av nymph activity) | (5/54) | (4/53) | (10/39) | (18/39) |
| Jun. – Sep. | $36.1 \pm 11.1ab$ | $55.1 \pm 11.7ac$ | $50.0 \pm 14.1bd$ | $83.7 \pm 10.3cd$ |
| (Av adult activity) | (26/72) | (38/69) | (24/48) | (41/49) |
| Oct. – Dec. 1996 | $51.9 \pm 13.6$ | $48.1 \pm 13.3$ | $58.3 \pm 16.1$ | $61.1 \pm 15.9$ |
| (Av nymph activity) | (27/52) | (26/54) | (21/36) | (22/36) |
| Sub-total | $32.6 \pm 6.9a$ | $38.6 \pm 7.2b$ | $44.7 \pm 8.5ac$ | $65.3 \pm 8.4bc$ |
| | (58/178) | (68/176) | (55/123) | (81/124) |

*Number positive samples per number tested samples. Percentage followed by the same letter within a row and treatment differ significantly (alpha 0.05 or greater).

*Period subsequent to suspension of acaricide application (March to December 96)*

Suspension of acaricide application was followed by an increase in the proportion of positive seroreactions in previously treated N'Dama and Gobra zebu cattle. In both previously treated and untreated animals the peak of positive seroreactions occurred during and subsequent to the period of activity of *A. variegatum* adults (June to September 96) (Table 2; Figures 1 and 2). In N'Dama cattle, similar cumulative proportions of positive seroreactions were observed in untreated ($38.6 \pm 7.2\%$) and previously acaricide-treated ($32.6 \pm 6.9\%$) animals. Conversely, in Gobra zebu cattle significantly higher ($P < 0.002$) antibody prevalence was detected in serum samples collected in previously untreated animals ($65.3 \pm 8.4\%$) compared to previously treated ($44.7 \pm 8.5\%$) cattle. Between-breed comparison revealed significantly lower ($P < 0.05$ or greater) prevalence of positive serological reactions in both untreated and previously treated N'Dama than in corresponding Gobra zebu cattle. The period March to May was characterised by low *A. variegatum* nymph and adult burdens in both previously treated and untreated cattle of both breed (mean nymph and adult burdens lower than one tick per animal) (Figure 2). In that period, significantly higher ($P < 0.001$) antibody prevalence was observed in untreated Gobra ($46.2 \pm 15.6\%$) in comparison with corresponding N'Dama cattle ($7.5 \pm 7.1\%$). All other tested comparisons were not statistically significant. In contrast, during and immediately following the period of abundance of *A. variegatum* adults, i.e. June to September 96 (mean adult burdens in previously untreated and acaricide-treated Gobra and N'Dama cattle were, 14.4, 8.9, 12.5 and 9.6, respectively), previously untreated N'Dama and Gobra zebu showed significantly higher ($P < 0.04$ or greater) antibody prevalence ($55.1 \pm 11.7\%$ and $83.7 \pm 10.3\%$ in N'Dama and Gobra cattle, respectively) compared with corresponding previously treated cattle ($36.1 \pm 11.1\%$ in N'Damas and $50.0 \pm 14.1\%$ in Gobras). Higher antibody prevalence was also observed both in untreated and previously treated Gobra zebu in comparison with corresponding N'Dama treatment groups. In the subsequent period (October to December 1996), characterized by a relative abundance of *A. variegatum* nymphs, differences within-breed and between-breed in proportions of seroreactors were not significant (Table 2).

## Discussion

Significant seasonal variations in prevalence of antibody against *C. ruminantium* were observed in N'Dama and Gobra zebu cattle maintained without

acaricide treatment, with peaks occurring during and immediately following the periods of abundance of *A. variegatum* adults. Conversely, investigations carried out in a similar ecoclimatic environment in neighbouring Senegal reported a stable and high proportion of positive seroreactors, approaching 95%, in the adult cattle population independent of *A. variegatum* instar occurrence (Gueye *et al.*, 1993). These contrasting findings might be explained by the specificity and/or sensitivity of the different tests used. Sensitivity and specificity of the MAP1-B antigen-ELISA used in the present study are estimated to be, for cattle, 96.5% and 99.7%, respectively (van Vliet *et al.*, 1996; Mondry *et al.*, 1998). On the contrary, the indirect immunofluorescent antibody assay employed by Gueye *et al.* (1993) suffers from poor specificity as it cross-reacts with several *Ehrlichia* spp. (van Vliet *et al.*, 1995; Jongejan and Bekker, 1999), known to occur in Senegal. Transmission of *Ehrlichia* spp. can also be accomplished by *Hyalomma* spp. and *Rhipicephalus* spp. ticks (Morel, 1981), reported to infest cattle in The Gambia and whose seasonal dynamics differ from that of *A. variegatum* adults (Mattioli *et al.*, 1997).

A progressive decrease in antibody titres resulting, consequently, in reduced *C. ruminantium* seroprevalence was observed in cattle reared under strict tick control in heartwater endemic areas (Norval *et al.*, 1995). Similarly, in our study, the significantly lower *A. variegatum* adult burdens in acaricide-treated animals (Mattioli *et al.*, 1998) produced a lower proportion of positive *C. ruminantium* seroreactions in comparison to untreated cattle throughout the period of tick control.

In The Gambia, mortality due to heartwater was recently reported in trypanosome infected Gobra zebu cattle (Mattioli *et al.*, 1994). The serological results, reported here, indirectly corroborate the presence of *C. ruminantium* in this country (Mattioli *et al.*, 1994). They also suggest adult *A. variegatum* are the main vectors in cattle. Moreover, although wide seasonal fluctuations in seroprevalence occurred both in untreated cattle and in intensively treated animals after the suspension of acaricide application, no overt cases of heartwater were observed (Mattioli *et al.*, 1998). In this context, the question arises whether a situation of endemic stability in cattle populations of different breeds can be estimated purely and solely through relative antibody prevalence, as the presence of natural resistance, as postulated to exist in N'Dama cattle (Mattioli *et al.*, 1994; Mattioli and Dempfle, 1995), could also affect transmission of tick-borne pathogens (Francis and Little, 1964). This argument deserves further debate and investigations. In addition, presence of antibodies is not necessarily associated to protective immunity to *C. ruminantium* infection (Lawrence *et al.*, 1993). However, Koney and Morrow (1990) observed lower susceptibility of the N'Dama breed, relative to

European and European × zebu cattle, to *A. variegatum*-associated dermatophilosis. Maillard *et al.* (1993a,b) postulated that resistance to dermatophilosis in creole zebu cattle is modulated by the presence of N'Dama genes in their genome, providing additional evidence for the possible existence of a genetic based resistant trait to *A. variegatum*-associated pathogens in N'Dama
cattle.

In the 1991 Livestock Sector Review (quoted in Mattioli *et al.*, 1997), improvement of milk production is identified as a major objective in The Gambia. Milk yield in Gambian N'Dama cows is known to be low (Agyemang *et al.*, 1991). Therefore, a continuous Jersey × N'Dama and Holstein × N'Dama crossbred cattle scheme has been set up at ITC with the aim to increase milk yield in the peri-urban production system. However, exotic × local crossbred cattle are more susceptible to heartwater than indigenous animals (Gueye *et al.*, 1982). In this respect, additional studies are needed in order to obtain more information on disease risk, assessment of presence or absence of endemic stability, and impact of heartwater in indigenous and crossbred cattle populations in The Gambia, with a view to designing appropriate tick control schemes adapted to different breeds of domestic ruminants and various production systems.

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