Chapter 2

Point seroprevalence survey of *Ehrlichia ruminantium* infection in small ruminants in The Gambia

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Clinical and Diagnostic Laboratory Immunology **12**: 508-512 (2005)

Abstract

Using the MAP1-B ELISA, we tested 1318 serum samples collected from sheep and goats at 28 sites in the 5 divisions of The Gambia to determine the *Ehrlichia ruminantium* seroprevalence rates and to assess the risk for heartwater. About half (51.6 %) of 639 sheep were positive, with seroprevalence rates per site varying between 6.9 % and 100 %. The highest seroprevalence was detected in the western part of the country (88.1 % in Western Division and 62.1 % in Lower River Division). Sheep in the two easterly divisions (Central River and Upper River Division) showed the lowest seroprevalence of 29.3 % and 32.4 % respectively, while those in North Bank Division showed an intermediate prevalence of 40.6 %. In goats, less than one third (30.3 %) of 679 animals tested were positive. The highest seroprevalence was detected in goat in North Bank Division (59 %) and Western Division (44.1 %). Goats in the Lower River Division showed an intermediate level of 21.9 %, whereas the lowest rates were found in the eastern part of the country (4.8 % in Central River and 2.3 % in Upper River Division). At nearly all sites, seroprevalence rates were higher in sheep than in goats. The results show a gradient of increasing heartwater risk for susceptible small ruminants from the east to the west of The Gambia. These findings need to be taken into consideration when future livestock upgrading programs are implemented.

Introduction

Heartwater (cowdriosis) is a major tick-borne disease of ruminants caused by the rickettsia Ehrlichia ruminantium and is transmitted by ticks of the genus *Amblyomma*. *Amblyomma variegatum* is the major vector in West Africa (22) and is distributed throughout most of sub-Saharan Africa and occurs on some islands in the Caribbean (23). Heartwater represents a major constraint to improvement of the livestock industry in sub-Saharan Africa. In The Gambia and neighboring Senegal, serological evidence of a high prevalence of *E. ruminantium* infection has been reported in cattle (8, 10, 17), which indicates a potential risk of heartwater disease for susceptible livestock. In contrast to indigenous cattle, which appear more resistant to heartwater (6, 16), the disease has been known in small ruminants in The Gambia as 'fayo' referring to cases of sudden death, characteristic of acute forms of the disease (23). Mortality occurs in indigenous local dwarf sheep and goats and is estimated at 10 % in endemic areas of the country (R. Mattioli and J. Jaitner, unpublished data). In addition, frequent occurrences of sudden death due to heartwater have been observed in indigenous sheep and goats following translocation from east to west of the country (B. Faburay, unpublished observation). These observations suggest the existence of a gradient of heartwater disease risk for susceptible livestock species and the possibility that a significant proportion of the small ruminant population in the east of the country has not been exposed to *E. ruminantium* infection. We carried out a countrywide serological survey to determine the distribution of *E. ruminantium* infection in small ruminants and to assess the heartwater risk for susceptible livestock.

Materials and Methods

Survey

Serum samples were collected from Djallonké sheep and West African Dwarf goats of both sexes in a cross-sectional study at 28 sites in all 5 divisions of The Gambia: Western, Lower River, North Bank, Central River and Upper River Divisions (Figure 1). The sites were located in three main agro-ecological zones; Sudanian, Sudano-Sahelian and Sahelian. The sites were chosen in consultation with livestock assistants based on owner cooperation and accessibility to the animals. Adult animals between 1 to 3 years were sampled in April 2004. All animals were maintained under a traditional husbandry system without acaricide treatment. Blood samples were kept on ice and serum was separated after 2 to 4 hours by centrifugation and stored at –20°C until use. A total of 1318 indigenous small ruminants comprising 639 sheep and 679 goats were sampled. The sites were selected in a transect layout to make the results representative of the country. The numbers sampled at different sites for each species are shown in Table 1. The largest number of sampling sites was in Western Division as this area is experiencing an expansion of livestock upgrading program carried out by the Gambian Government in collaboration with the

International Trypanotolerance Centre (ITC), involving cattle breeds (Holstein and Jersey) highly susceptible to heartwater disease.

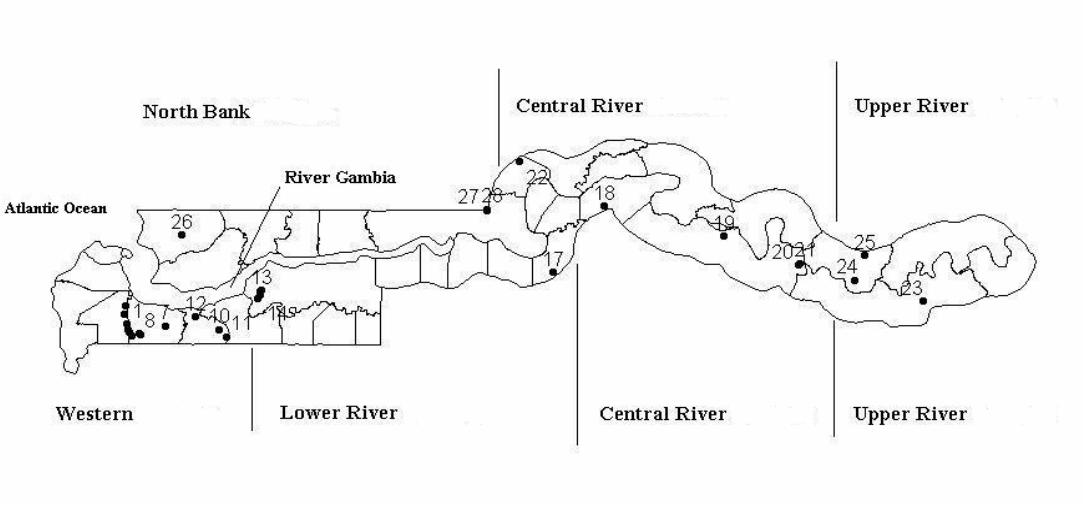

Figure 1. Map of The Gambia showing the five divisions and the distribution of sampling sites.

MAP1-B ELISA

The indirect MAP1-B ELISA, based on a recombinant truncated form of the Major Antigenic Protein 1 of *E.ruminantium,* was carried out as described previously (18, 21). Although the assay does not detect antibodies to ehrlichial agents infecting domestic ruminants such *E. bovis* and *E. ovina*, antibodies are detected against *E. canis* (which infects dogs) and *E. chaffeensis* (a human pathogen) (21). The MAP1-B ELISA has been shown to perform satisfactory for small ruminants (5, 15) with a specificity of 98.9 % and 99.4 % for caprine and ovine sera respectively (19, 21). Each serum sample was tested in duplicate. For sheep, each test included duplicate negative control sera obtained from a heartwater-naïve sheep of the Tesselaar breed. Duplicate positive control sera were obtained from Tesselaar sheep #229 infected with the Gambian isolate Kerr Seringe 1 of *E. ruminantium* at the Faculty of Veterinary Medicine, Utrecht, The Netherlands. This stock of *E. ruminantium* was isolated from a naturally infected goat in the Gambia. In goats, each MAP1-B ELISA test also included duplicate positive control sera which were obtained from a Saanen goat infected with the Senegal isolate of *E.ruminantium* at Utrecht University. The negative control serum was obtained from the same animal prior to infection. Species-specific second step IgG antibodies conjugated with horse-radish peroxidase were used.

The cut-off point for the ELISA was determined by addition of 2 standard deviations (SD) to the mean OD values of reference local non-infected sheep (n = 24) and goat (n = 18) populations (18). The sheep and goat populations were considered negative based on the following: (i) they originated from northern Senegal which is known to be free from *Amblyomma* ticks; and ii) they were highly susceptible to *E. ruminantium* infection as demonstrated by a high rate of mortality due to confirmed cases of heartwater upon first exposure to *A.variegatum*-infected ticks under

natural conditions in an enzootic area (7). The cut-off point for sheep was determined at 0.53 (SD = 0.10) and for goats as 0.58 (SD = 0.11). OD values of samples that were equal to or greater than the cut-off value were considered positive for *E. ruminantium* infection. Variations between OD values of duplicate negative control sera or between duplicate positive control sera on each plate were acceptable only if such variations were lower than 10%.

Statistical analysis

Plate-to-plate variation was considered by a statistical test for significance in difference, using the General Linear Model (SAS), among OD values of the positive controls included in each plate. Variation among the positive controls for the accepted plates was not significant for sheep (P = 0.4457; Coefficient of Variation = 14.7 %) or for goats (P = 0.4514; Coefficient of Variation = 11.8 %). The mean OD values of the positive controls included in the accepted plates was 1.5 ± 0.22 for sheep and 1.49 ± 0.18 for goats. Comparison for statistical significance of differences in the proportion of *E. ruminantium*-seropositive samples was carried out at two levels: (i) between species within a division using the Wilcoxon two-sample test and (ii) between divisions cumulatively (sheep and goats combined) and within species using the General Linear Model procedure (SAS Statistical Programme) and Kruskal-Wallis one-way analysis of variance respectively.

Results

Of the 639 sheep samples tested, 51.6 % were positive for *E. ruminantium* infection with seroprevalence at individual sites ranging from 6.9 % to 100 % (Table 1). The highest seroprevalence was seen in the two westerly divisions, Western (88.1%) and Lower River (63.1 %) (Table 2). Sheep populations in the two easterly divisions, Central River (29.3 %) and Upper River (32.4 %) showed the lowest levels of *E. ruminantium* seroprevalence, whereas animals sampled in North Bank Division (40.6 %) showed an intermediate level of seroprevalence (Table 2). In contrast to the results for sheep, of the 679 goat samples collected, only about one third (30.5 %) were positive for *E. ruminantium* infection (Table 1). Overall, the highest seroprevalences were detected in goat populations in North Bank (59 %) and Western Divisions (44.1 %) with more than half of the animals sampled in North Bank Division testing positive for *E.ruminantium* (Table 2).

TABLE 1. *Ehrlichia ruminantium* seroprevalence in sheep and goats at 28 different sites in The Gambia

Division	Village	Site	Seroprevalence (%)		
			Sheep	Goats	Total
Western Division	Basori	1	75(8)	51.6(31)	56.4(39)
	Berefet	12	90.3(31)	31.7(60)	51.6(91)
	Bitta	11	94.7(38)	0(12)	72(50)
	Duwasu	8	88.9(9)	33.3(9)	61.1(18)
	Giboro Kuta	3	81.8(33)	47.8(46)	62(79)
	Gida	2	100(2)[c]	42.1(19)	47.6(21)
	Jenunkunda	9	100(2)[c]	45.5(11)	53.8(13)
	Mandinaba	5	85.7(7)	70(10)	76.5(17)
	Somita	10	75(16)	15.2(33)	34.7(49)
	Talokoto[b]	4	-	80(5)	80(5)
	Toubakuta[b]	6	-	77.8(9)	77.8(9)
	Toumani Tenda	7	100(14)	68(50)	75(64)
Lower River Division	Bodeyel	17	30.9(55)	20(5)	30(60)
	Burong	16	100(9)	42.9(7)	75(16)
	Julakunda	13	100(14)	0(12)	53.8(26)
	Missira	15	100(13)	40(5)	83.3(18)
	Taborongkoto	14	85(20)	33.3(3)[c]	78.3(23)
Central River Division	Jimballa K/Chendu	22	41(61)	7.7(52)	25.7(113)
	Mamutfana	18	6.9(72)	0(20)	54.3(92)
	Sare Sofie	21	31.3(16)	0(8)	20.8(24)
	Sinchan Faranba	20	29.6(27)	1.5(69)	9.4(96)
	Yorro Beri Kunda	19	100(12)	17.7(17)	51.7(29)
Upper River Division	Kulkullay	23	22.2(54)	4.2(48)	13.7(102)
	Missira Sandou	24	47.6(42)	0(20)	32.3(62)
	Sare Demba Torro	25	26.7(15)	0(18)	12.1(33)
North Bank Division	Kolli Kunda	26	68.8(16)	48.7(37)	54.7(53)
	Mbappa Ba	27	50(16)	50(22)	50(38)
	Mbappa Mariga[a]	28	24.3(37)	73.2(41)	50(80)

[a]Village with a high introgression of Sahelian sheep genes into the local population
[b]No sheep were sampled in Talokoto and Toubakuta villages
[c]Prevalence was based on small sample size as there were very few animals presented for sampling

Seroprevalence in goats in Lower River Division (21.9 %) showed an intermediate level, with the two easterly divisions, Central River (4.8 %; range = 0 % to 17.7 %) and Upper River (2.3 %; range = 0 % to 4.2 %) showing the lowest level of seroprevalence (Table 2, Figure 2). In all divisions, except for North Bank Division, overall seroprevalence was significantly higher ($P < 0.001$) in sheep than in goats (Table 2). Moreover, at all sample sites (except Mbappa Mariga in North Bank Division), the proportion of seropositive samples was consistently higher in sheep than in goats. Differences observed in the proportion of *E. ruminantium*-positive samples among sheep populations in the different divisions of The Gambia were statistically significant ($P < 0.001$). The same conclusion applied to goats. Similarly, differences in the overall seroprevalence between divisions were statistically significant ($P<0.001$) (Table 2).

TABLE 2. Proportions of total small ruminant population in the five divisions of The Gambia and overall heartwater seroprevalence rates

Division	No. of sites	% of total livestock[a]			% of seropositive animals (total no. sampled)		Probability[b]
		Sheep	Goats	Total	Sheep	Goats	
Western Division	12	10.9	11.9	11.5	88.1 (160)	44.1 (295)	< 0.001
Lower River Division	5	7.9	11.8	10.2	63.1 (111)	21.9 (32)	< 0.001
North Bank Division	3	12.7	23.5	19.0	40.6 (69)	59.0 (100)	= 0.019
Central River Division	5	43.5	34.8	38.4	29.3 (188)	4.8 (166)	< 0.001
Upper River Division	3	25.0	18.0	20.9	32.4 (111)	2.3 (86)	< 0.001

[a]Deduced from reference 1
[b] Probability comparing differences between the proportion of seropositive sheep and seropositive goats in a Division (a *P*value of 0.05 or less is significant)

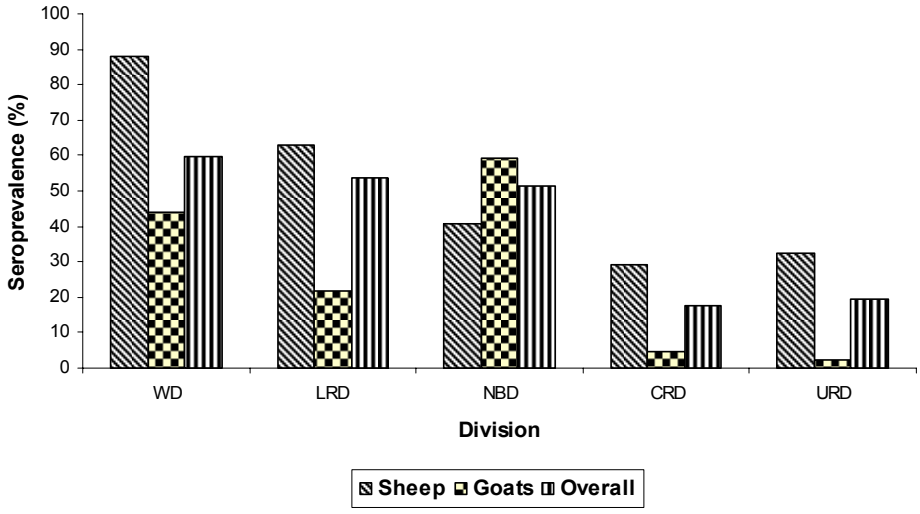

FIG. 2. Distribution of *E. ruminantium* infection in sheep and goats as determined by serology in the five divisions of The Gambia. **WD,** Western Division; **LRD,** Lower River Division; **NBD,** North Bank Division; **CRD**, Central River Division; **URD**, Upper River Division.

Discussion

Ehrlichia ruminantium seroprevalence in small ruminants was found to be highest in the western part of The Gambia with Western Division showing the highest prevalence of nearly 60 % and Lower River and North Bank Divisions showing sero-prevalences of more than 50 %. Although the two easterly divisions, Central River and Upper River, account for the highest proportions of the small ruminant population in The Gambia of 38.4 % and 20.9 % respectively (Table 2), overall seroprevalence of *E. ruminantium* in small ruminant populations in these regions was

significantly lower than the westerly divisions (Western, North Bank and Lower River; Figure 2). At most sites in Central River and Upper River Divisions, the serological prevalence was generally lower than 50 %, resulting in a substantial population of sheep and goats susceptible to heartwater.

In a recent study (E. Hoeven et al., unpublished data) a relatively high introgression of Sahelian genes was found in indigenous goats in Central River Division as opposed to those in Western Division. Interestingly, the easterly region accounts for over 60 % of the small ruminant population in The Gambia (Table 2), of which Sahelian sheep and goats and their crosses with local dwarf sheep and dwarf goats, constitute a significant proportion. Generally, Sahelian sheep and goats found in the easterly part of The Gambia originate from *Amblyomma*/heartwater-free areas in northern Senegal and are therefore susceptible to heartwater disease. However, due to their larger size, farmers in this region show preference for them and use them for crossbreeding with local sheep and goats. The above factors therefore suggest the existence of a lower risk of *E. ruminantium* infection in most of the eastern part of the country. Thus small ruminants, including those of susceptible Sahelian genotypes, may have a greater chance of survival in these areas. This, among other factors, possibly allowed the proliferation of large populations consisting of local dwarf sheep and goats, crossbred (Djallonké sheep/West African dwarf goat x Sahelian sheep/goats) as well as Sahelian small ruminants.

Heartwater, described as a case of sudden death in small ruminants, is perceived as a major problem by farmers in The Gambia. Our unpublished observations confirmed that small ruminants have suffered mortality due to confirmed cowdriosis after translocation from the Central River Division to the coast in Western Division.

Although possible antigenic variation between stocks of *E. ruminantium* (11, 12) in the different regions of The Gambia may be a possible cause of the mortalities (since there was no record on the immune status of the translocated animals), it is postulated that small ruminants that died from heartwater, after translocation from the east to the west of the country, constituted a naïve group that had no previous exposure to *E. ruminantium* infection. Furthermore, in a three-year period (1997-1999) of monitoring by post mortem of the major causes of mortalities in local dwarf sheep and goats at an ITC field station in Keneba in Lower River Division, heartwater, confirmed in brain-crush smears, accounted for 36 % of deaths in sheep and 25 % in goats (R. Mattioli and J. Jaitner, unpublished data). Analysis of similar data collected from October 1996 to January 1999 from local dwarf sheep and goats at the ITC Kerr Seringe station in Western Division, showed

deaths due to heartwater were 17.9 % in sheep and 12.5 % in goats. The higher seroprevalence in sheep observed in this study, combined with lower incidences of clinical disease in goats in The Gambia as indicated above, appears to agree with the observations made elsewhere in West Africa by Koney et al. (14) that local dwarf goats in Ghana are more resistant to heartwater than dwarf sheep. The higher seroprevalence in sheep could also be an indication that local dwarf sheep are more tolerant of *E. ruminantium* infection than dwarf goats or merely that sheep are more frequently infested with *A. variegatum* ticks than goats. A longitudinal study in Ghana of *E.ruminantium* seropositivity using a competitive ELISA (20) with sensitivity comparable to the MAP1-B ELISA (4), revealed high antibody levels detectable for longer periods in sheep than in goats (3). Therefore, it is also possible that the higher seroprevalence in sheep could be due to longer persistence of antibodies. Interestingly, although both sheep and goats are vulnerable to the disease, peracute cases of heartwater are reported to be more common in the latter (23). This requires further investigation.

Surveys of *E. ruminantium* seroprevalence in small ruminants have been carried out in other parts of Africa. In Ghana, Koney et al. (14) reported a seroprevalence of 51 % for sheep and 28 % for goats, figures that are comparable with those of 51.6 % for sheep and 30.3 % for goats in the present study. In north Cameroon, using a modified PC-ELISA, a mean *E. ruminantium* prevalence of 58-66 % was reported for sheep and 65-66 % for goats (1a). In a comparable study in southern Africa, using the MAP1-B ELISA, a lower *E. ruminantium* seroprevalence was detected in indigenous goats in the northern part of Mozambique (8.1 %) than in the southern part (65.6 %) resulting in mortalities due to heartwater after translocation of animals from the north to the south (2). These findings suggest that a substantial proportion of small ruminant populations in parts of heartwater-endemic areas in Africa are at risk.

However, serological cross-reactions between *Ehrlichia* species have also been reported. As far as the MAP1-B ELISA is concerned, the assay does not detect antibodies to known ehrlichial agents infecting domestic ruminants such *E. bovis* and *E. ovina*, but antibodies are detected against *E. canis* (which infects dogs) and *E. chaffeensis* (a human pathogen) (21). Although the MAP1-B ELISA has been shown to perform satisfactory for small ruminants, cross-reactions with unknown *Ehrlichia* species have been detected based on levels of seropositivity among sheep and goats in *Amblyomma*-free areas in South Africa and Zimbabwe (5, 13, 15). Similar *Ehrlichia* species of low pathogenicity may occur in the Gambia, since they have been found in neighboring Senegal (9). Such cross-reactions may falsely indicate previous exposure to heartwater, whereas

in fact such animals are highly susceptible and thereby underestimating the risk for small ruminants to contract the disease.

In conclusion, our serological results in conjunction with recorded cases of heartwater-related mortalities indicate that *E. ruminantium* is widespread in The Gambia and this poses a threat to susceptible livestock species. An estimated 50% of the sheep and 70 % of the goats have not been exposed to *E. ruminantium* infection, and constitute a group at risk from the disease. There appears to be a gradient of risk for livestock increasing from the eastern part of the country towards the western coastal region. This gradient may be positively correlated with the distribution of *A. variegatum* ticks on sheep and goats in the country, which showed a significantly lower abundance in the eastern part of the country (0.01 ticks per animal) than in the western part (0.76 ticks per animal) (B. Faburay et al., unpublished data). It is recommended that in future livestock upgrading programs in the Gambia susceptible small ruminants should be protected by prophylactic treatment using oxytetracyclines or vaccination using attenuated or inactivated rickettsiae, possibly in conjunction with tick control, prior to their translocation from the eastern to the western region of the country.

Acknowledgement

This work was supported by The European Development Fund, contract no REG/6061/006, project "Concerté de Recherche-Developpement sur l'Elevage en Afrique de l'Ouest" (PROCORDEL) and the International Foundation for Science (IFS), Stockholm, Sweden. We appreciate the support of the Utrecht Scholarship Programme and the ICTTD-2 concerted action project through the INCO-DEV programme of the European Commission under contract no. ICA4T-CT-2000-30006. We thank Dr. Kwaku Agyemang, Director General of ITC for his support. We are grateful for the support of Dr. Cornelis Bekker, Amar Taoufik, Saja Kora, Nerry Corr and Dr. M. Mbake. Prof. Gerrit Uilenberg is thanked for his critical comments on the manuscript.

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