# A contribution to the epidemiology of *Ehrlichia Ruminantium* infection (Heartwater) in small ruminants in The Gambia

Animal Health Research Working Paper 4

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## EXECUTIVE SUMMARY

The rapid growth in human population in The Gambia and sub-Saharan Africa generally, has spurred an increased demand for protein of animal origin. Ruminant livestock, especially small ruminants, play a central role as an important source of animal protein and income for the vast majority of the rural farming community. In The Gambia, and the subregion of West Africa as a whole, improvement of the livestock industry, which is dominated by ruminant livestock species, to meet the growing challenge of food and nutritional security is severely constrained by animal diseases, especially heartwater. Efforts to improve domestic milk and meat production through introduction of more productive but highly susceptible exotic ruminant livestock from temperate countries as well as from heartwater-free regions in Africa have been severely hampered by the incidence of the disease. Heartwater, considered to be the most important tick-borne infection in West Africa, is the cause of an estimated mortality rate of 10 % in indigenous small ruminant breeds in The Gambia (unpublished information) and more than 50 % in exotic ruminant livestock introduced from heartwater-free areas to endemic areas (Uilenberg, 1983). Because women especially in rural areas own and manage more than 60 % of small ruminants in The Gambia (Jaitner et al., 2001), the impact of heartwater on the welfare of rural households and communities is therefore significant.

The control of heartwater depends on a combination of chemotherapy, vector control by use of acaricides and immunisation using Infection and Treatment method (practised principally in southern Africa). The administering of the most effective drug (oxytetracycline), even if available, to control heartwater, is constrained by the acute nature of the disease in ruminants, which does not always allow timely intervention to prevent a fatal outcome of infection. Additionally, the drugs are expensive for resource-poor farmers. The application of acaricides by regular spraying or dipping of livestock is quite effective in preventing the disease, however, the chemicals are expensive and their use causes environmental concerns and possible contamination of the food chain. In

addition, the problem is complicated by the ability of the ticks to develop acaricide resistance. Immunisation by use of a protective vaccine would appear to be the most effective control measure, but cannot be routinely applied due to enormous antigenic and genotypic diversity among *E. ruminantium* strains resulting in lack of protection between heterologous strains. To overcome this formidable obstacle, there is need for a better understanding of the epidemiology of heartwater.

In The Gambia, no systematic epidemiological investigation of heartwater has ever been done and thus this study, the first of such nature, was initiated by ITC and has been carried out over a 3-year period starting in 2001. The study used improved diagnostics, which have superior sensitivity and specificity and thus provided a better insight into:

- the distribution of heartwater infection in The Gambia
- the genotypic diversity of heartwater agent in The Gambia
- the efficiency of the candidate vaccine against heartwater in The Gambia

Notwithstanding the shortcomings of MAP1 B ELISA reported in various serological surveys, we used the assay, because it so far demonstrated to be the most sensitive and specific test for serodiagnosis of heartwater in small ruminants. Serological evidence showed that throughout the country about half of the sheep and 70 % of the goat populations have not been exposed to *E. ruminantium* infection, and constitute a high-risk group for heartwater disease. There is also a gradient of heartwater-risk for susceptible livestock species, with risk increasing from the eastern region of the country towards the western region to the coast. Although antigenic disparity between stocks of *E. ruminantium* in the different regions of the country could be an important factor, significant difference in the seroprevalence as reported also in Mozambique, could be the cause of mortalities in small ruminants upon translocation from low disease-risk areas (from the eastern region of the country) to high-risk areas (the western region of the country). Heartwater, at present, is perceived as a major problem in

sheep and goats. The high prevalence of infection in the western regions of the country (Western and North Bank and Lower River Divisions) poses a serious threat to future livestock upgrading programmes in the country. Since differences in ecological parameters among the various regions could result in potential immunotypic differences, a study was carried out to genetically characterise potential stocks of heartwater agent at selected sites located in the major agroecological zones of the country as a basis for further immunological studies.

Thus restriction enzyme analysis was carried out to elucidate possible genotypic diversity. Comparison of the restriction profiles of the *map*1 genes of *E. ruminantium* in tick and animal samples originating from sites located in the major agroecological zones of The Gambia indicated that more than one genotype of *E. ruminantium* exist in The Gambia. The greatest diversity of profiles was observed in samples originating from the Sudano-Guinean (Coastal Gambia) and Western Sudano-Sahelian (mid Gambia) zones, which was attributed to introduction of carrier animals from different geographical areas. However, genotypic and immunotypic diversity do not necessarily correlate among stocks of *E. ruminantium*. We therefore evaluated the capacity of the current inactivated vaccine candidate (Gardel isolate) to protect sheep against heartwater caused by the local isolate. This commenced with the initial isolation, for the first time in The Gambia, of one local heartwater agent (Kerr Serigne isolate). The result showed lack of full protection and was attributed to possible antigenic disparities between the two isolates. The results attained so far indicated that there is need for further epidemiological investigation of heartwater in The Gambia. Given the role of ruminant livestock in poverty alleviation and especially in meeting food and nutritional security in The Gambia, the search for a vaccine that would significantly reduce mortalities especially in small ruminant populations should continue and be supported.

# I. BACKGROUND

Heartwater (Cowdriosis) is a major tick-borne disease of ruminants caused by the rickettsia *Ehrlichia* (formerly *Cowdria*) *ruminantium* (Dumler et al., 2001) and is transmitted by ticks of the genus *Amblyomma*. The most widespread vector is *Amblyomma variegatum*, which is distributed in the Caribbean and all over sub-Saharan Africa except certain areas of southern Africa (Uilenberg, 1983).

Heartwater represents a major constraint to improvement of the livestock industry in The Gambia and sub-Saharan Africa as a whole. In The Gambia, small ruminants, which are predominantly managed by resource-poor rural women, are especially susceptible to infection and suffer high mortalities. Another important aspect of the disease is that its presence severely limits the capacity to introduce high-yielding cattle and small ruminants into endemic areas.

The significance of cowdriosis for domestic livestock production is demonstrated by recorded cases of mortalities in indigenous traditionally managed small ruminants (shown in Appendix 1). Cases of mortality have also been reported in animals translocated from heartwater-free (northern Senegal) or less-endemic (eastern region of The Gambia) areas to areas of high endemicity in the Western region of the country. A similar occurrence has been reported in Mozambique, where mortality associated with the disease, was observed in indigenous small ruminants after translocation (Bekker et al., 2001) and mass restocking (Hanks et al., 1998) from the northern part of the country characterized by low seroprevalence of *E. ruminantium* determined by MAP-1B ELISA, to locations with high seroprevalence in the south of the country (Bekker et al., 2001). Importantly, in both The Gambia and neighbouring Senegal, serological evidence of high prevalence of *E. ruminantium* infection has been reported (Mattioli et al., 2000; Gueye et al., 1993a, 1993b). Furthermore, mortality due to heartwater has been reported in Gobra zebu cattle undergoing *Trypanosoma congolense* experimental infection (Mattioli et al., 1994), in Holstein x N'Dama crossbred

calves (unpublished data) and in small ruminants. Consequently, the disease is a potential obstacle to the development of the smallholder peri-urban dairy sector based on the exploitation of the genetic superiority of the F1 crosses between the highly susceptible Holstein breed and the N`Dama.

A cost-effective and sustainable strategy to control heartwater requires the use of a protective vaccine. However, implementation of any large-scale vaccination campaign is dependent on understanding the diversity of the disease-causing agent. Previous studies have indicated the existence of remarkable genotypic (Allsopp et al., 1997) and immunotypic (determined in cross-protection studies) (Jongejan et al., 1991) diversity among isolates from geographically close as well as widely separate areas. This represents a major complicating factor in the development of a universally protective vaccine against the disease.

In this context and in the absence of meaningful epidemiological information, we initiated a systematic epidemiological investigation into heartwater in The Gambia with a view to recommending and developing a control measure. The objectives of the research were to carry out:

1. A serological transect study of *E. ruminantium* infection in small ruminants in The Gambia

2. Genetic characterisation of *E. ruminantium* in small ruminants and ticks in The Gambia

3. Evaluation of protection of small ruminants against cowdriosis using a candidate inactivated vaccine

## II. METHODOLOGY

A systematic epidemiological study of heartwater in small ruminants in The Gambia was carried out. The study involved a serological transect study to determine the distribution of risk of heartwater disease using the MAP1 B

ELISA; genetic characterisation of *Ehrlichia ruminantium* in small ruminants and ticks from the different agroecological zones of The Gambia; isolation of a local stock of *E. ruminantium* in bovine aortal endothelial cell culture with subsequent evaluation for cross-protection in a vaccination trial.

The serological transect study was carried out in five regions of The Gambia (Figure 1), Western Division (WD), Lower River Division (LRD), North Bank Division (NBD) Central River Division (CRD) and Upper River Division (URD). Genetic characterisation was done based on the potential influence of ecological variability on genetic diversity of microbial or disease causing agents, and was carried out at sites in the three main agroecological zones of The Gambia: Sudano-Guinean zone, around coastal Gambia, Western Sudano-Sahelian zone, located in Lower River Division and Eastern Sudano-Sahelian zone, located in Central River Division. In this study, the Sudano-Sahelian zone, which covers most territory of The Gambia, was divided into 2 zones, the Western and the Eastern zones due to the remarkable diversity in ecological parameters described below.

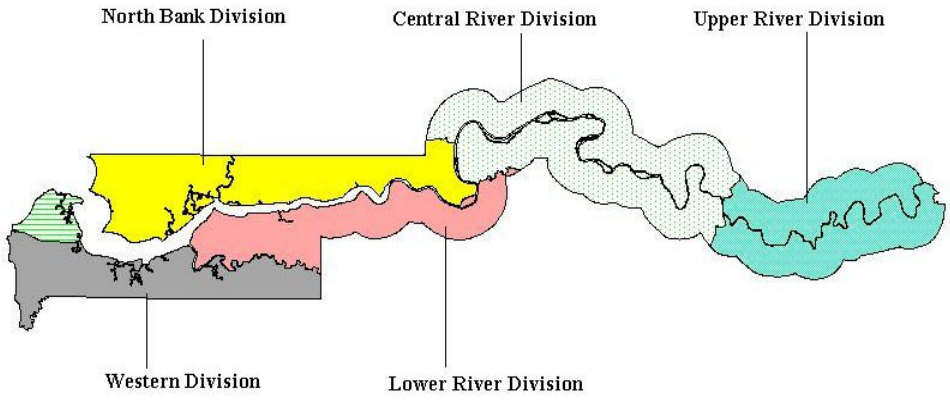


Figure 1. Map of The Gambia showing the 5 major Regions where the study was conducted

These zones are characterized by unimodal rainfall, which occurs from June to October. The Sudano-Guinean zone lies within the 900 and 1210 mm of rainfall isohyets. Maximum daily temperatures range from 26° C to 28° C (Climatological Unit, Dept. of Water Resources) and the vegetation is predominantly savannah-woodland or woodland in certain areas. Annual precipitation in the Western Sudano-Sahelian zone averages 800 mm of rainfall isohyets with maximum daily temperatures ranging from 28 to 38° C. The vegetation is composed predominantly of degraded savannah woodlands interspersed with unimproved grasslands and farmlands. Annual precipitation in the Eastern Sudano-Sahelian zone averages 700 mm of rainfall isohyets with maximum temperatures ranging from 30 to 40° C. The vegetation is mainly open savannah interspersed with trees, grasses and farmland.

## 2.1 Serological transect study of *Ehrlichia ruminantium* infection in small ruminants in The Gambia

### 2.1.1 Sample sites and sampling of animals

Serum samples were collected from Djallonke sheep and West African Dwarf goats of both sexes in April 2004, in a cross-sectional study at 28 sites in the 5 regions of The Gambia (Figure 2): Western Division, Lower River Division, North Bank Division, Central River Division and Upper River Division. The sites were chosen with the assistance of resident Livestock Assistants based on owner cooperation and accessibility to the animals. Adult animals were sampled and most animals were within the age range of 1 year to 3 years with 6 months being the minimum age-limit. All animals were maintained under traditional husbandry system without acaricide treatment; depending on the village/sample site, all sheep and goats were allowed to wander freely around the homestead for feed and food residues and/or graze in the bush during the day. At night the animals were penned in sheds or barns. Blood samples were collected in plain vacutainer tubes by venipuncture. The samples were transported on ice to the laboratory and serum was separated after several hours by centrifugation. The serum samples were then kept as aliquots in cryotubes at –20 °C until use.

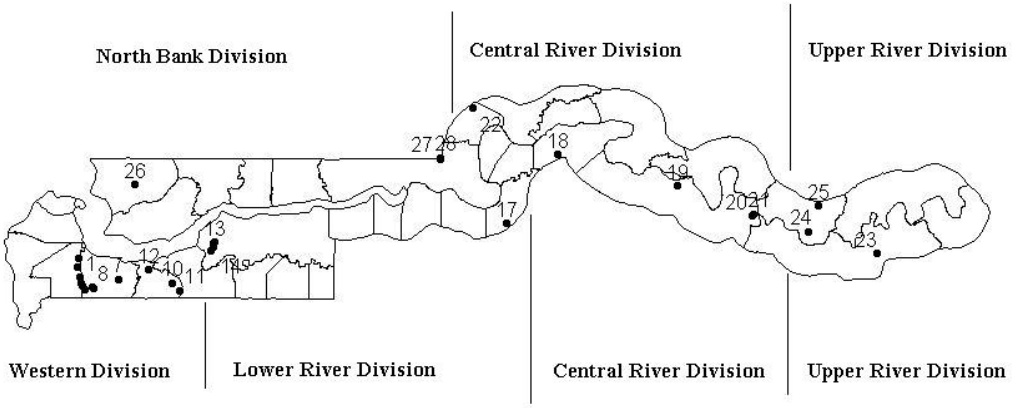


Figure 2. Map of The Gambia showing Regions and the distribution of sampling sites (Sites 1-12 in the Western Division, sites 13-17 in Lower River Division, sites 18-22 in Central River Division, sites 23-25 in URD and sites 26-28 in NBD)

2.1.2 ELISA

The indirect MAP1 B ELISA was carried out as described by Mboloi et al. (1995). For testing sera from each of the species (sheep and goats), specific antispecies, i.e., anti-sheep/goat IgG (H + L)/PO conjugate was used. Optical densities (OD) of the ELISA tests were measured using Titertek Multiskan® ELISA reader (Titertek, Flow Laboratories Inc.) using a 405 nm wavelength. Each serum sample was tested in duplicate. Each test included a duplicate negative and positive control. The cut-off value (COV) was determined by addition of 2 standard deviations to the mean optical density value of a reference negative local sheep and goat population (Mboloi et al., 1999). OD values of samples that were equal to or greater than the COV were considered positive for *Ehrlichia ruminantium* infection.

## 2.2 Genetic characterisation of *E. ruminantium* in small ruminants and ticks in The Gambia

2.2.1 Preparation of genomic DNA from animals and ticks and PCR analysis

Buffycoat was prepared from EDTA blood collected from traditionally managed local dwarf sheep and dwarf goats at sites located in the three agroecological zones. The blood was filled into plain capillary tubes and centrifuged for 5 minutes to separate the buffycoat. The microtube was cut just beneath the buffycoat. The latter was applied on Whatman® filter paper Nr. 3 or 4 by bringing the tube into contact with the filter paper to have it absorbed. This was allowed to dry at room temperature.

DNA from animals was extracted by Modified Plowe extraction on buffycoats using Saponine/Chelex. Details of the extraction protocol will be provided in subsequent publications.

*A. variegatum* ticks collected at the same study sites located in the different agroecological zones were stored in 70 % ethanol until further analysis. DNA was extracted from them using the modified protocol of QiaAmp DNeasy Tissue kit (Westburg, Leusden, The Netherlands). The genomic DNA extracts were tested using a semi-nested *map*1 PCR: The first amplification was done using the forward primer ERF3 and the reverse primer ERR1. The semi nested second amplification was carried out using the forward primer ERF3 and the reverse primer ERR3 (primer sequences unpublished). The PCR amplified a 720-738 bp fragment of the *map*1 gene of *E. ruminantium*. Amplification products of samples that gave positive bands on 2 % agarose gels were used for further characterisation using restriction fragment length polymorphism (RFLP) assay.

2.2.2 Restriction fragment length polymorphism

We initially evaluated several restriction enzymes (*Tsp* R1, *Tsel*, *Msp*1, *Alu*1) to determine the characteristics of their restriction profiles of the *map*1 gene. The *Alu*1 restriction enzyme was selected as the most suitable. The semi-nested *map*1

PCR products were digested with this enzyme in buffer NE 2 using 4 µl of amplified DNA product in a 15 µl total reaction volume. The restricted samples were then loaded onto a 10 % polyacrylamide gel. A 100 bp ladder was included to determine the fragment size. DNA fragments were electrophoresed and then subjected to silver staining.

## 2.3 Evaluation of protection of small ruminants against cowdriosis using an inactivated vaccine

### 2.3.1 Isolation of the Gambian stock (Kerr Serigne) of *E. ruminantium*

#### *2.3.1.1 Origin of the isolate*

The infective material of the first Gambian isolate of *E. ruminantium* was prepared from a local West African Dwarf goat #1946 just before death in the form of a blood stabilate. The goat had its origin in the Sudano Guinean zone (SG) in coastal Gambia. The animal prior to death, manifested symptoms characteristic of heartwater and examination of the Giemsa-stained brain smear confirmed the presence of elementary bodies in vascular endothelial cells.

#### *2.3.1.2 Blood stabilate preparation*

Jugular blood was collected into EDTA vacutainer tubes in December 2001. The blood was chilled to 4 °C and DMSO added drop wise to 10 % v/v while stirring over crushed ice. The blood-DMSO mixture was immediately divided into subsamples and kept frozen at –80 °C.

The aliquots were shipped under cold chain about a week later to the Institute of Tropical Medicine (ITM) in Antwerp, Belgium. An aliquot of one of the infective stabilates was inoculated into goat #4639 in which it caused clinical heartwater. Infective blood stabilate was subsequently prepared and aliquoted as above and stored in dry ice.

In July 2002, 2 ml of the infective blood stabilate prepared from goat #4369 was taken to Utrecht University, The Netherlands, on dry ice where it was first stored at –80 °C and then snapfrozen in liquid nitrogen the following day. In June 2003,

the stabilate was inoculated into sheep #229 of the Tarselaar breed. The stabilate caused hyperthermia in the sheep in first passage and the profile of the temperature reaction is shown below (Figure 3). The first batch of infective blood stabilate was prepared as described previously when the temperature of the sheep reached 40 °C on day 8-post inoculation (15 June) and a second batch was prepared similarly including samples of plain EDTA blood on day 9-post infection (16 June) at 41.5 °C pyrexia (Figure 3). The DMSO blood stabilate was aliquoted in 2 ml tubes and initially stored at –80 °C and later snap frozen in liquid nitrogen for subsequent use in a cross-protection vaccination trial in The Gambia. The infective plain EDTA blood was used to inoculate bovine aortal endothelial cell culture for isolation.

Additionally, we sub-inoculated sheep #239 with 2 ml of the infective blood stabilate prepared from sheep #229. The former similarly responded with pyrexia on day 8-post infection. Both animals were later treated with 6 ml of 10 % oxytetracycline LA.

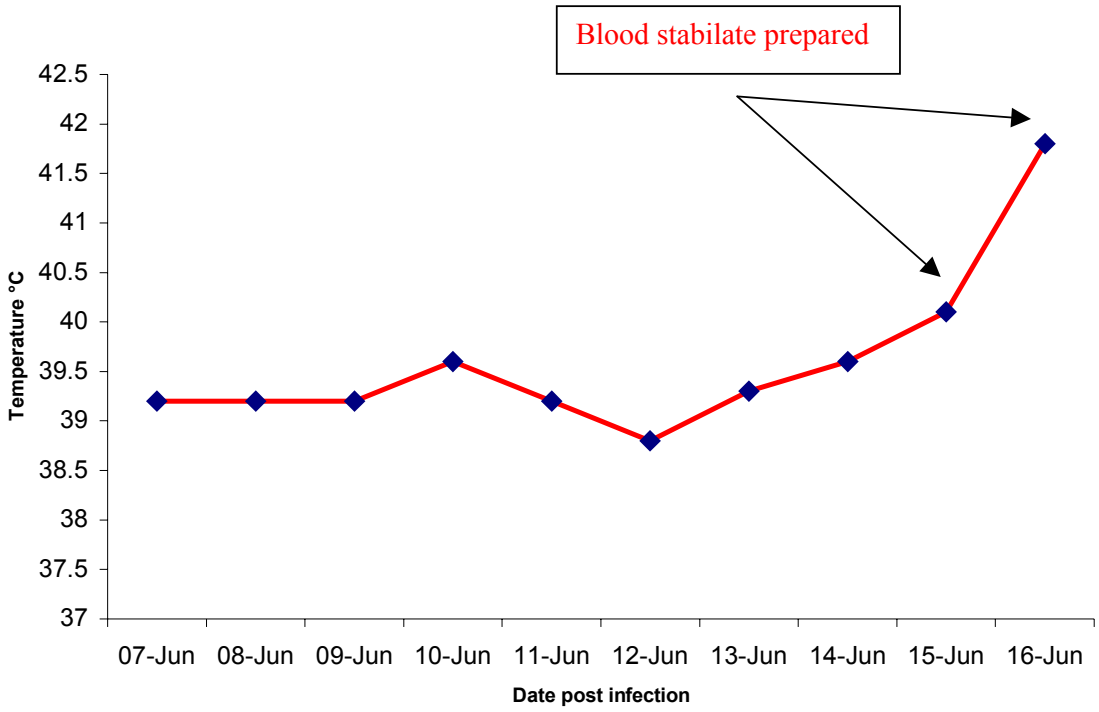


Figure 3. Temperature dynamics of sheep #229 after needle infection with infective blood stabilate

### 2.3.1.3 In vitro cultivation of the Gambian stock of *Ehrlichia ruminantium* in bovine aortal endothelial (BAE) cells

The cells (BAE) were grown in BHK21 medium in 25 $cm^2$ culture flasks according to standard protocol. Infection of confluent cultures was initiated by washing the cultures with Hank´s BSS and then inoculated with infective EDTA blood prepared on day 9-post infection from sheep #229.

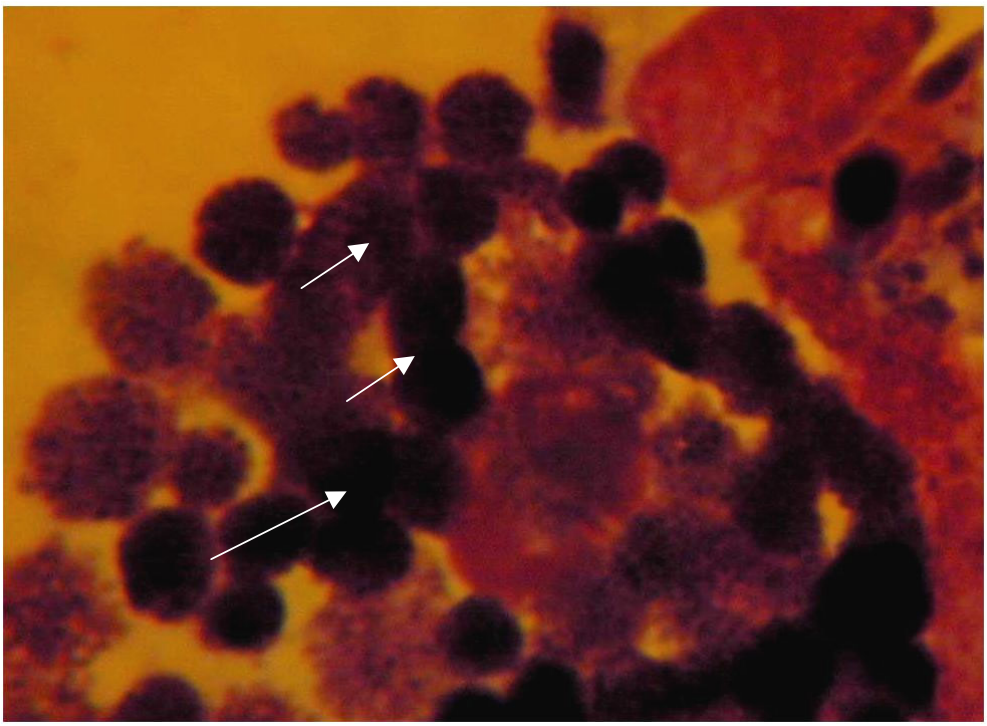

Figure 4. Morulae and elementary bodies of *Ehrlichia ruminantium* (Kerr Serigne isolate) in bovine aortal endothelial (BAE) cell culture

The cultures were regularly monitored and when the monolayer showed signs of infection about 3 weeks later, some cells were put on microscopic slides and stained with Giemsa (Figure 4). A sample of the isolate gave positive signals in a PCR assay with *map*1 primers (not shown) at Utrecht University (using a sample from cell culture) and at ITM (using blood sample). Since the stock of *Ehrlichia ruminantium* originated from Kerr Serigne (location of the International

Trypanotolerance Centre) in the Sudano-Guinean agroecological zone, the isolate was named Kerr Serigne (KS).

### 2.3.2 Cross-protection studies

#### *2.3.2.1 Experimental animals*

Fourteen Sahelian sheep, originating from *Amblyomma* and heartwater-free areas in northern Senegal were used for the experiment. The animals were tested by MAP1 B ELISA to confirm their seronegative status and they proved to be negative for antibodies against heartwater. Subsequently, the animals were divided into 2 groups of 7 and were all dewormed using albendazole about 3 weeks prior to the start of the experiment.

#### *2.3.2.2 Preparation of inactivated vaccine*

A vaccine made from *E. ruminantium* organisms of Gardel isolate of Guadeloupe was tested for its crossprotective capacity against challenge using the Gambian Kerr Serigne isolate. The vaccine isolate was bulk-produced from cell culture at IBET, Lisbon, Portugal. Prior to administering the vaccine, the stock was diluted with PBS as recommended. The preparation was then emulsified in an equal volume of the adjuvant Montanide ISA 50V (Seppic, Paris, France) as per recommendation.

#### *2.3.2.3 Vaccination of sheep with the inactivated vaccine and challenge*

Animals of group 1 constituting the experimental group were vaccinated subcutaneously with 2 ml of the inactivated vaccine containing the Gardel strain, whereas the control group received placebo. One month after first vaccination, animals in the experimental group were given a second booster vaccination of 2 ml of the same inactivated vaccine. Pre-vaccination sera as well as sera at 2 weeks following first and second vaccination were collected from all animals to assess seroconversion. About 3 weeks after the second vaccination, animals in both groups were challenged intravenously with 2 ml of infective blood stabilate containing the Kerr Serigne isolate of *E. ruminantium*.

*2.3.2.4 Monitoring of infection status*

All animals were kept in an insect and tick-proof stable throughout the experiment at ITC. Rectal temperatures were recorded daily for each animal after challenge infection to detect the onset and duration of the clinical infections. A rectal temperature reading of 40 °C was considered significant. The incubation period, time to death and number of deaths were recorded for each group of animals. When death occurred, a post-mortem examination was performed to establish the cause of death. Animals that die of heartwater infections typically show hydropericardium, hydrothorax and ascites. The brain was removed and brain-crushed smears were prepared for identification of *E. ruminantium* colonies inside brain capillary endothelial cells (Purchase, 1945). The experiment ended about 3 weeks post challenge infection.

*2.3.2.5 Detection of antibody responses and rickettsaemia*

Detection of antibodies was done to confirm exposure to the vaccine antigen. Sera were prepared from the animals pre-vaccination and 2 weeks following each vaccination to detect the development of antibodies to *E. ruminantium* antigens by MAP1-B ELISA (Van Vliet et al., 1995). A sample of buffycoats was collected at random from 5 animals (#1838, #1827, #1835, #1828, #1834) during febrile reaction (41 °C) and tested in semi-nested pCS20 PCR assay to determine infection status.

2.4 Statistical analysis

Comparison for statistical significance of differences in the proportion of *E. ruminantium*-seropositive samples was carried out at two levels: between species within a region using the Wilcoxon two-sample test, and between regions cumulatively (sheep and goats) and within species using General Linear Model procedure (SAS Statistical Programme) and Kruskal-Wallis one-way analysis of variance respectively.

The ability of the vaccine to protect against heartwater was tested statistically by the General Linear Model using SAS system (SAS Institute Inc.), comparing the

mortality or survival rates of vaccinated and control groups. The time difference of the incubation periods and the duration to death between experimental and control groups were similarly tested statistically.

## III. RESULTS AND DISCUSSION

### 3.1 Serological transect study of *Ehrlichia ruminantium* infection in small ruminants in The Gambia

#### 3.1.1 Sampling

A total of 1318 indigenous small ruminants comprising 679 sheep and 639 goats were sampled in 5 regions of The Gambia (Figure 1 and 2). The numbers sampled at the different sites for each of the species is shown in Table 1. At all the sites, the number of sheep sampled ranged from 2 to 72 except for 2 villages (Talokoto and Toubakuta) in Western Division where no animals were sampled. For goats the sample size per site ranged from 3 to 60. The greatest number of sampling sites was in Western Division as the Region experiencing an increased expansion of the F1 crossbreeding programme involving breeds (Holstein and Jersey) highly susceptible to heartwater disease.

#### 3.1.2 Seroprevalence in sheep

Table 1 shows the seroprevalence of *E. ruminantium* infection in sheep and goats and the number of samples collected per site and per species in The Gambia. The distribution of seroprevalence of *E. ruminantium* in sheep and goats overall and from each region of The Gambia is shown in Figure 5. Of the 639 sheep samples collected about half (51.6 %) of them were positive for *E. ruminantium* infection with seroprevalence ranging from 6.9 % to 100%. The highest seroprevalence was seen in the two most westerly regions, Western Division (88.1%) and Lower River Division (63.1 %) shown in Table 2 and Figure 5. Sheep populations in the two easterly regions, Central River and Upper River Divisions showed the lowest levels of *E. ruminantium* serpoprevalence of 29.3 % and 32.4 % respectively. Sheep sampled in North Bank Division showed an intermediate level of seroprevalence (40.6%).

### 3.1.3 Seroprevalence in goats

In contrast, of the 679 goat samples collected, less than half (30.3 %) of them were positive for *E. ruminantium* infection. Overall, the highest seroprevalences were detected in goat populations in North Bank Division (59 %) and Western Division (44.1 %) with more than half of the animals sampled in North Bank Division testing positive for heartwater infection (Table 2). Seroprevalence in goats in Lower River Division (21.9 %) showed an intermediate level, with the two most easterly Regions, Central River Division (4.8 %; range = 0 % to 17.7 %) and Upper River Division (2.3 %; range = 0 % to 4.2 %) showing the lowest level of seroprevalence.

### 3.1.4 Comparison of seroprevalence between species and Regions

The number of seropositive sheep and seropositive goats sampled at locations in each of the Regions were compared statistically using Wilcoxon two-sample test. In addition, differences for statistical significance between Regions but within species were tested using Kruskal-Wallis one –way analysis of variance. General Linear Model (SAS statistical programme) was used to test differences in overall (sheep and goats combined) seroprevalence between the Regions. In all Regions, except for North Bank Division, overall seroprevalence was significantly higher ($P < 0.001$) in sheep than in goats (Table 2). In fact at all sites except for one (Mbappa Mariga) in North Bank Division, the proportion of seropositive samples was consistently higher in sheep than in goats (Table 1). The lower *E. ruminantium* seroprevalence in sheep relative to goats in North Bank Division was attributed to increasing introgression of Sahelian genes in local sheep population through crossbreeding with local breeds for sale during the Muslim feast 'Tobaski'. On the scale of preference among farmers in The Gambia, sheep ranked higher than goats (Bennison et al., 1997) and production of crossbred animals by farmers is also practised more frequently in the former species (Jaitner et al., 2001). This, against the background of improved management results in lower exposure of sheep to infective tick challenge.

**Table 1. *Ehrlichia ruminantium* seroprevalence in sheep and goats at sites in The Gambia, (and the number of samples collected per site)**

| Region | Village | Seroprevalence (%) | | |
|---|---|---|---|---|
| | | Sheep | Goats | Total |
| Western Division | Basori | 75.0(8) | 51.6(31) | 56.4(39) |
| | Berefet | 90.3(31) | 31.7(60) | 51.6(91) |
| | Bitta | 94.7(38) | 00.0(12) | 72.0(50) |
| | Duwasu | 88.9(9) | 33.3(9) | 61.1(18) |
| | Giboro Kuta | 81.8(33) | 47.8(46) | 62.0(79) |
| | Gida | 100.0(2) | 42.1(19) | 47.6(21) |
| | Jenunkunda | 100.0(2) | 45.5(11) | 53.8(13) |
| | Mandinaba | 85.7(7) | 70.0(10) | 76.5(17) |
| | Somita | 75.0(16) | 15.2(33) | 34.7(49) |
| | Talokoto* | - | 80.0(5) | 80.0(5) |
| | Toubakuta* | - | 77.8(9) | 77.8(9) |
| | Toumani Tenda | 100.0(14) | 68.0(50) | 75.0(64) |
| Lower River Division | Bodeyel | 30.9(55) | 20.0(5) | 30.0(60) |
| | Burong | 100.0(9) | 42.9(7) | 75.0(16) |
| | Julakunda | 100.0(14) | 00.0(12) | 53.8(26) |
| | Missira | 100.0(13) | 40.0(5) | 83.3(18) |
| | Taborongkoto | 85.0(20) | 33.3(3) | 78.3(23) |
| Central River Division | Jimballa Kerr Chendu | 41.0(61) | 7.7(52) | 25.7(113) |
| | Mamutfana[1] | 6.9(72) | 00.0(20) | 54.3(92) |
| | Sare Sofie | 31.3(16) | 00.0(8) | 20.8(24) |
| | Sinchan Faranba | 29.6(27) | 1.5(69) | 9.4(96) |
| | Yorro Beri Kunda | 100.0(12) | 17.7(17) | 51.7(29) |
| Upper River Division | Kulkullay | 22.2(54) | 4.2(48) | 13.7(102) |
| | Missira Sandou | 47.6(42) | 00.0(20) | 32.3(62) |
| | Sare Demba Torro | 26.7(15) | 00.0(18) | 12.1(33) |
| North Bank Division | Kolli Kunda | 68.8(16) | 48.7(37) | 54.7(53) |
| | Mbappa Ba | 50.0(16) | 50.0(22) | 50.0(38) |
| | Mbappa Mariga | 24.3(37) | 73.2(41) | 50.0(80) |

[a]Village with a high introgression of Sahelian sheep genes into the local population; *No sheep were sampled in Talokoto and Toubakuta villages

**Table 2. Proportions of total small ruminant population in the five regions of The Gambia, and heartwater seroprevalence in these regions**

| Region | No. of sites | Proportion of total livestock* | | | Proportion of seropositive (total number sampled) | | |
|---|---|---|---|---|---|---|---|
| | | Sheep | Goats | Total | Sheep | Goats | Probability |
| 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 |
| Overall | 28 | 100 % | 100 % | 100 % | 51.6 % (639) | 30.3 % (679) | < 0.001 |

*Deduced from 2000/2001 National Agricultural Sample Survey (Statistical Yearbook of Gambian Agriculture: 2000)

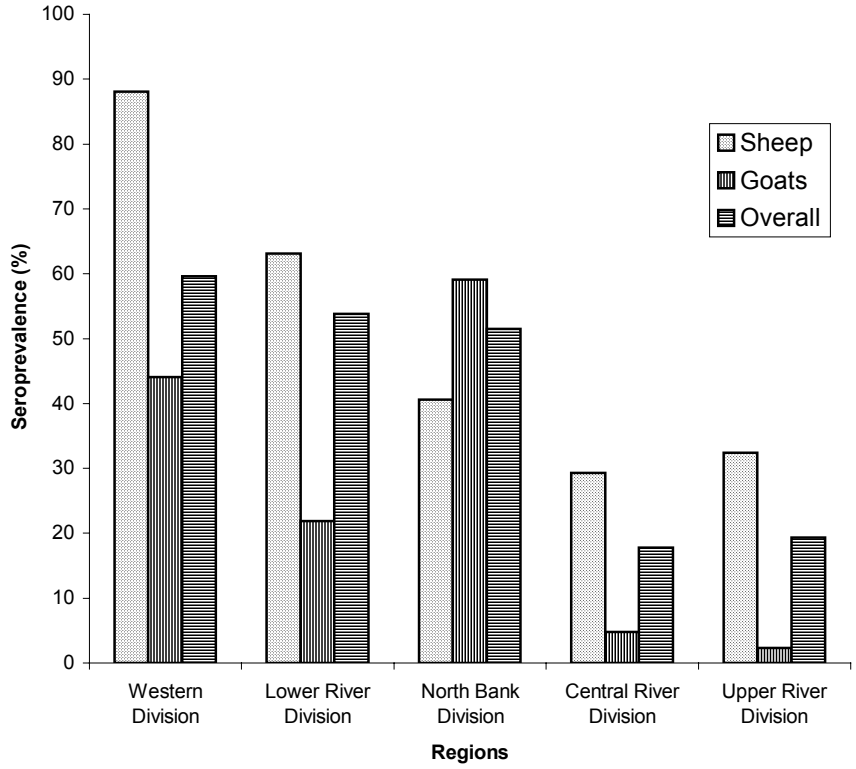


Figure 5. Distribution of seroprevalence of *E. ruminantium* infection in sheep and goats in the five regions of The Gambia

Differences observed in the proportion of *E. ruminantium*-positive samples between sheep populations in the different Regions of The Gambia were statistically significant ($P < 0.001$). The same conclusion applied for goat populations. Similarly, differences in the proportion of overall seroprevalence in Western Division (59.6 %, $n = 455$), Lower River Division (53.8 %, $n = 143$), North Bank Division (51.5 %, $n = 169$), Central River Division (17.8 %, $n = 354$) and Upper River Division (19.3 %, $n = 197$) were statistically significant ($P < 0.001$).

### 3.1.5 Discussion

The countrywide survey of seroprevalence of *E. ruminantium* described in this report is the first such cross-sectional study undertaken for heartwater generally and specifically in small ruminants in The Gambia. We used the MAP1 B

ELISA, which is highly specific for *E. ruminantium* (van Vliet et al., 1995) in comparison to other serological assays, in this survey. Importantly, the assay, considered having low sensitivity for bovine sera (Semu et. al., 2001; Peter et al., 2002; Bell-Sakyi et al., 2003), shows high sensitivity for ovine and caprine sera (Van Vliet et. al., 1995; Mahan et al., 1998; De Waal et al., 2000). Overall, *E. ruminantium* seroprevalence in small ruminants was found to be highest in the westerly regions of The Gambia with Western Division showing the highest prevalence of close to 60 % and Lower River and North Bank Divisions showing seroprevalences of more than 50 %. Although, the two easterly regions, Central River and Upper River Divisions, account for the highest 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, 17.8 % and 19.3 % respectively (Figure 5). This presented a picture of a gradient in risk of heartwater disease, with increasing trend, going from the eastern region of the country towards the west, for susceptible livestock species. Small ruminants particularly those belonging to the International Trypanotolerance Centre (ITC) suffered mortality due to confirmed cases of cowdriosis after translocation from the field station in Central River Division to the main station on the coast in Western Division (unpublished information). In Mozambique, similar occurrence was reported in indigenous small ruminants after translocation (Bekker et al., 2001) and mass restocking (Hanks et al., 1988) from the northern part of the country characterized by low seroprevalence of *E. ruminantium* infection determined by MAP-1B ELISA, to locations with high seroprevalence in the south of the country (Bekker et al., 2001). Although possible antigenic variation between stocks of *E. ruminantium* (Jongejan et al., 1988) in the different regions of The Gambia may play a role, it is recommended that susceptible sheep and goats should be subjected to prophylactic treatment/vaccination prior to their translocation from the eastern region of the country characterized by low seroprevalence to the western region with high seroprevalence; if this measure proved unfeasible they should be prevented from tick infestation.

*Amblyomma variegatum* is the commonest vector, at least in two of the study locations (Mattioli et al., 1993, 1995, 1998), in Western and Lower River Divisions. However, this tick species was found to be the least abundant of the four main genera of ixodid ticks (*Rhipicephalus, Hyalomma, Boophilus, Amblyomma*) at a site in the eastern region of the country (Mattioli et al., 1997). Although the latter study was limited in terms of area-coverage, the results, given the near-uniformity of the bioclimatic environment, seemed reflective of the population structure of the different tick genera in that region of the country. At most sites in these regions (CRD and URD), the serological prevalence was generally lower than 50 %, resulting in a substantial population of sheep and goats susceptible to heartwater. A recent study (Leak et al., unpublished information) reported a relatively high introgression of Sahelian genes in indigenous goat populations in the Central River Division as opposed to those in Western Division. Interestingly, the easterly region (CRD and URD combined) accounts for over 60 % of the small ruminant population in The Gambia. The above evidences therefore suggest the existence of a lower risk of heartwater in most parts of the easterly region of the country, resulting in the proliferation, additionally, of non-pure (Djallonke sheep/WAD goat x Sahelian sheep/goats) as well as Sahelian small ruminant genotypes.

Heartwater in The Gambia has been perceived as a major problem in small ruminants for a long time. However, the absence of systematic studies and lack of diagnostic capacities in field laboratories upcountry resulted in lack of records on the true mortality due to the disease in small ruminants. In a three-year (1997-1999) monitoring of the major causes of mortalities in local dwarf sheep and dwarf goats by post mortem at an ITC field station in Lower River Division, heartwater accounted for 36 % of deaths in sheep and 25 % in goats (unpublished information, see annex). Analysis of similar data collected from October 1996 to January 1999 from local dwarf sheep and dwarf goats at ITC Kerr Seringe station in Western Division, showed deaths due to heartwater were 17.9 % in sheep and 12.5 % in goats. The concept of endemic stability

may not be valid for management of heartwater in small ruminants as it is in cattle (H.R. Andrew, personal communication 1992).

Host preference of *Amblyomma* ticks shows that adult instars are less likely to be found on small ruminants than on cattle. The former are predominantly parasitized by immatures, which would result in a different transmission pattern and affect endemic stability (Yunker, 1996). Thus young animals born outside the period of peak occurrence of nymphs are less likely to be exposed to infective tick challenge during their brief period of reduced susceptibility to clinical disease, which is necessary for subsequent immunity (Norval et al., 1992; Perry and Young, 1995). Consequently, this creates a population that is susceptible to heartwater disease and could possibly explain the occurrence of mortality in indigenous sheep and goats in endemic areas. The present study revealed a higher seroprevalence of *E. ruminantium* in sheep than in goats at all (except one site Mbappa Mariga, Table 1) sites. In agreement with Koney et al. (2004, in press), the higher seroprevalence in sheep observed in this study, combined with lower incidences of clinical disease in goats as indicated above, suggests that local dwarf goats may be more resistant to heartwater than dwarf sheep.

Thus throughout the country about half of the sheep and 70 % of the goat populations have not been exposed to *E. ruminantium* infection, and constitute a group at risk of heartwater disease. There is also a gradient of heartwater-risk for susceptible livestock species with risk increasing from the eastern region of the country towards the western region to the coast. Although the disease is at present perceived as a major problem in sheep and goats, the high seroprevalence in the westerly regions of the country, Western and North Bank and Lower River Divisions, poses a threat to future livestock upgrading programmes in the country. The former two regions are the current targets for expansion of the F1 crossbreeding scheme for milk production based on the exploitation of high productivity trait of susceptible exotic breeds of cattle

(Holstein and Jersey). Generally, animals of non-African origin are highly susceptible to heartwater and other tick borne infections (Camus et al., 1996; Koney, 1995).

### 3.2 Genetic characterisation of *E. ruminantium* in small ruminants and ticks in The Gambia

We reported on the *Alu*1 restriction profiles of *map*1 genes of *E. ruminantium* extracted from *A. variegatum* ticks and small ruminants. The *map*1 gene of Crystal Springs, an *E. ruminantium* isolate from Zimbabwe, was also restricted and included on the gel for comparison. It showed that the *map*1 profile of Kerr Serigne (KS), the Gambian isolate, is distinctly different from that of Crystal Springs (figure not shown). The *map*1 profile of the Senegal isolate was also distinctly different from the KS isolate (not shown). One of the samples from the ESS zone showed a combined profile of KS and ESS profile #3 (not shown).

Further analysis of the restriction profiles of the *map*1 gene of samples originating from sites in different agroecological zones of the country indicated the potential presence of at least 8 different genotypes of *E. ruminantium* (Table 3). We detected 4 and 5 different *map*1 profiles in the SG and WSS zones respectively.

Table 3. RFLP restriction profile analysis of *map*1 gene from *A. variegatum* ticks and animals in different agroecological zones (aez)

| Profile number | Frequency | Sample ID | Origin of sample (aez) |
|---|---|---|---|
| 1 | 1 | 19 | SG |
| 2 | 2 | 61, [a]KS | WSS, SG |
| 3 | 6 | 85, 62, 63, 54, 90, [a]K4315 | WSS, WSS, WSS, WSS, ESS, WSS |
| 4 | 1 | 1320 | SG |
| 5 | 1 | 81 | WSS |
| 6 | 2 | 80, 163 | WSS, ESS |
| 7 | 1 | 135 | SG |
| 8 | 1 | [a]L4306 | WSS |

[a]Samples originating from animals

Some of the *map*1 profiles originating from sites in different agroecological zones showed similarity. Specifically, the profile of KS (Kerr Serigne) isolate, which originated from the SG zone, was identical to the profile of sample 61 of profile #2 originating from the WS zone (Table 3). The remaining 3 profiles, #1 and #4 were entirely distinct and showed no similarity with any of the profiles in the different zones. Of the 5 different profiles characterised in the WSS zone, 3 showed identity with profiles in SG (profile #2 mentioned above) and ESS zone (profile #3 and #6). The latter zone had 2 different profiles, which showed no uniqueness and were identical to profiles (#3 and #6) in the WSS zone (Table 3).

It is thus postulated that the Kerr Serigne stock, isolated in the SG zone was introduced from the WSS zone, whereas the 2 profiles (#3 and #6 in the WSS zone) are ´genotypes´ introduced from the ESS zone following the trade route of livestock in The Gambia. Interestingly, the WSS zone is host to an ITC station for Open Nucleus Ruminant Pure Breeding Programme characterised by regular introduction of breeding stock from diverse geographical areas. Among samples originating from the SG zone, we found 4 different profiles, of which 3 were unique, #1, #4, #7 (Table 3). Additionally, the profile of the Kerr Serigne isolate was distinctly different from Crystal Springs (not shown). The remarkable diversity of profiles in this zone was attributed to the effect of introduction of carrier animals from other parts of the country and beyond its borders. In this zone, which encompasses the coastal area, is located the largest center for trade in ruminant livestock in the country. The results so far suggest that multiple genotypes of *E. ruminantium* exist in The Gambia.

### 3.3 Evaluation of protection of small ruminants against cowdriosis using an inactivated vaccine

#### 3.3.1 Detection of antibody responses and rickettsaemia

Pre-vaccination antibody response showed that all animals were seronegative for cowdriosis as determined by MAP1-B ELISA. Antibody response to *E.*

*ruminantium* antigen at 2 weeks after the first vaccination regimen indicated 5 out of 7 animals in the vaccinated group seroconverted. Animals #1829 and #1841 did not seroconvert. However, at 2 weeks post-booster vaccination, 6 of the 7 animals seroconverted including animal #1841, whereas animal #1829 remained seronegative. The reason for the non-seroconversion of animal 1829 could not be explained. The antibody response demonstrated that the respective animals were exposed to the vaccine antigen. All the 5 animals tested in the semi-nested pCS20 PCR assay showed positive rickettsaemia during febrile reaction (not shown).

3.3.2 Protective capacity of the inactivated vaccine

Table 4 summarizes the results of the vaccination trial using the Kerr Seringe isolate (Figure 4) as challenge material. There were no significant differences ($P$ = 0.7830) between incubation periods in the vaccinated and control groups. The mean incubation period in the vaccinated group was 11.3 days whereas it was 11.7 days in the control group.

Comparison of days to death between groups revealed no significant difference ($P$ = 0.3097) between the vaccinated and control groups. The mean number of days to death was 9 for the vaccinated group and 10.7 for the control group (Table 4).

Table 4. Clinical reactions of sheep challenged with Kerr Serigne isolate

| Sheep number | Group | Incubation period (days) | Maximum temp (°C) | Time to death (days) | Outcome |
|---|---|---|---|---|---|
| 1827 | Exp | 10 | 41.3 | - | Survived |
| 1828 | Exp | 10 | 41.1 | 9 | Fatal heartwater |
| 1829 | Exp | 16 | 41.4 | 10 | Fatal heartwater |
| 1830 | Exp | 10 | 41.0 | 10 | Fatal heartwater |
| 1835 | Exp | 10 | 40.3 | - | Survived |
| 1838 | Exp | 11 | 40.9 | 7 | Fatal heartwater |
| 1841 | Exp | 12 | 41.3 | - | Survived |
| 1826 | Cont | 10 | 41.5 | 14 | Fatal heartwater |
| 1831 | Cont | 14 | 41.5 | 9 | Fatal heartwater |
| 1832 | Cont | 14 | 41.2 | 8 | Fatal heartwater |
| 1834 | Cont | 10 | 41.4 | 11 | Fatal heartwater |
| 1836 | Cont | 8 | 40.8 | 14 | Fatal heartwater |
| 1837[1] | Cont | - | 39.9 | - | Survived |
| 1840 | Cont | 14 | 40.5 | 8 | Fatal heartwater |

[1]Sheep #1837 did not respond to the challenge inoculum

In determining the protective efficacy of the vaccine against the local challenge material, a survival rate of 42.9 % (3 out of 7 animals survived) was recorded in the vaccinated group, whereas 1 out of 7 animals, representing a survival rate of 14.3 %, survived. There was no significant difference ($P$ = 0.2707) in the survival rate between the vaccinated and control groups. Furthermore, all the animals that received the vaccine reacted to challenge with an elevated temperature of a little above 41 °C (except animal #1835 = 40.3 °C; #1838 = 40.9 °C). This is a manifestation of lack of full immunity (partial immunity) specifically in animals #1827, #1835 and #1841 that survived challenge infection (Table 4). All deaths were due to heartwater and were confirmed by post-mortem examination and identification of colonies of *E. ruminantium* in the brain smears. It was evident from this study that the inactivated Gardel vaccine prepared in Montanide ISA 50V did not fully protect sheep against heartwater caused by the Kerr Serigne isolate, albeit the lack of statistically significant difference in survival rate between the vaccinated and the control groups. The lack of complete protection of the Gardel inactivated vaccine against the Kerr Serigne isolate probably resulted from disparities in antigenic composition between these two *E. ruminantium* isolates.

Indeed, lack of cross-protection between *E. ruminantium* isolates has been attributed to the antigenic differences (Van Winkelhoff & Uilenberg, 1981; Jongejan et al., 1991; Mahan et al., 2001).

## IV. CONCLUSIONS

It is evident that heartwater is endemic in The Gambia and poses a risk to susceptible livestock species introduced particularly from heartwater-free areas. Small ruminants have particularly shown to suffer higher mortalities than cattle. Throughout the country about half of the sheep and 70 % of the goat populations have not been exposed to *E. ruminantium* infection, and constitute a group at risk for heartwater disease. Importantly, there exists a gradient of heartwater-risk for susceptible livestock species with risk increasing from the eastern region of the country towards the western region to the coast. This poses a potential threat to movement or introduction of susceptible livestock species from heartwater-free or less endemic areas. It is necessary therefore that any development strategy geared towards improving the performance of the livestock industry in The Gambia takes into consideration the development of a sustainable control measure for heartwater.

The development of a protective vaccine appears to be the most cost-effective and sustainable control strategy. Antigenic diversity among the various isolates of the heartwater agent, however, poses an obstacle to attaining this objective. Genetic characterisation undertaken in this study showed the existence of different genotypes, which is also highly indicative of different immunotypic strains, in The Gambia. Thus a stock of heartwater agent has been isolated for the first time in The Gambia and was subsequently evaluated against a culture-derived inactivated vaccine, containing the Gardel strain from Guadeloupe, for cross-protection against heartwater in sheep. The vaccine was shown to be protective against homologous challenge in various trials on Guadeloupe. Our results indicated a protective efficacy (survival rate) of 42.9 % in the vaccinated group against 14.3 % in the control group. Although the differences between the two groups were not statistically significant including a confidence interval that includes zero, the results indicated that the vaccine is not fully protective against challenge with the local stock (Kerr Seringe) isolate. Moreover, three of the vaccinated animals survived challenge infection (by hyperthermia) thus manifesting partial immunity. The lack of full

protection by the vaccine was attributed to disparities in antigenic composition between the two isolates. These findings suggest the need for further epidemiological studies of heartwater in The Gambia and as well the need to isolate additional strains of the heartwater agent existent in different agroecological zones with subsequent characterisation in cross-protection studies. The outcome of these epidemiological studies especially in field situations should be based on the use of improved diagnostics and it is thus recommended that future studies should evaluate the comparative performance of the different molecular assays in these areas. Given the importance of heartwater to food and nutritional security especially for women and children in The Gambia, these research efforts need to be given all the necessary support.

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**Appendix 1. Proportion of Giemsa-stained brain smears from indigenous Djallonke sheep and West African Dwarf goats positive for cowdriosis (ITC purebreeding flock in Keneba)**

| Animal | Year | Period | Proportion of brain samples | | | |
|---|---|---|---|---|---|---|
| | | | T/samples[a] | Erpos[b] | Others[c] | % positive |
| **Sheep** | 1997 | Jan. – Dec. | 25 | 16 | 9 | 64 |
| | 1998 | Jan.- Dec. | 76 | 19 | 57 | 25 |
| | 1999 | May – Nov. | 12 | 6 | 6 | 50 |
| **Total** | | | **113** | **41** | **72** | **36** |
| **Goats** | 1997 | Jan. – Dec. | 40 | 21 | 19 | 53 |
| | 1998 | Jan. – Dec. | 116 | 18 | 97 | 16 |
| | 1999 | May – June | 2 | 0 | 2 | 0 |
| **Total** | | | **158** | **39** | **119** | **25** |

T/samples[a] = total brain samples examined
Erpos[b]= *Ehrlichia ruminantium*-positive
Others[c] = deaths related to pneumonia, coccidiosis, enteritis, mange and accident