Chapter 7

7.1 General Discussion

This thesis described the first systematic epidemiological investigation into heartwater in The Gambia. Prior to this study, information on the presence and distribution of the disease in the country was fragmentary and based on clinical observations in afflicted animals without definitive post mortem diagnosis. The absence of systematic studies and lack of diagnostic capacities in field laboratories upcountry resulted in lack of records on the impact of the disease in small ruminants. The only available reports on the occurrence of heartwater in domestic ruminants in The Gambia were based on results of *post mortem* examinations of brain-crushed smears for *E. ruminantium* elementary bodies from fatal cases in local dwarf sheep and dwarf goats of the experimental flock of the International Trypanotolerance Centre in Kerr Seringe and Keneba (Faburay et al., 2004). Moreover, mortality due to cowdriosis was observed in experimentally *Trypanosoma congolense*-infected Gobra zebu cattle in The Gambia (Mattioli et al., 1994). This was further investigated in a seroprevalence study for *E.ruminantium* in cattle subjected to acaricidal treatment (Mattioli et al., 2000). These reports confirmed the endemicity of heartwater in The Gambia and indicated that small ruminants were more susceptible to the disease than cattle (Mattioli et al., 1994; Osaer and Goossens, 1999) . In order to control heartwater in susceptible livestock in The Gambia, especially in smallholder traditional livestock husbandry systems, it is important to understand the epidemiology of the disease, particularly on the prevalence and distribution of infection in the target population.

The MAP1-B ELISA reported to be suitable to detect antibodies to *E. ruminantium* infection in ovine and caprine sera was used to assess the level and distribution of heartwater-risk in The Gambia in a countrywide point seroprevalence survey involving 1318 local dwarf sheep and dwarf goats (De Waal et al., 2000; Mahan et al., 1998b). The survey (**Chapter 2)** showed that *E. ruminantium* infection is widespread in The Gambia, although an estimated 50 % of sheep and 70 % of goats had not been exposed to the infection and therefore constitute a group at risk from the disease. In addition, it showed a gradient of heartwater disease risk for livestock increasing from the eastern part of the country to the western part towards the coastal region. Our unpublished observations confirmed that small ruminants have suffered mortality due to confirmed cowdriosis after translocation from the eastern part of the country to the western part on the coast. It is

hypothesized 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.

Surveys of *E. ruminantium* seroprevalence in small ruminants have been carried out in other countries in sub-Saharan Africa which also demonstrated varying levels of heartwater risk (Awa, 1997; Bekker et al., 2001; Bell-Sakyi et al., 2004; Koney et al., 2004). The disease risk gradient in The Gambia appears to correlate positively with the distribution of *A. variegatum* ticks on sheep and goats in the country; small ruminants in the eastern part of the country carried a significantly lower tick burden per animal than their counterparts in the western part of the country. Gueye et al. (1993) reported a similar correlation in indigenous cattle in Senegal. The comparatively low disease risk in the eastern part of the country appeared to favour the proliferation of large populations of small ruminants consisting of local dwarf sheep and goats, crossbred (Djallonké sheep/West African dwarf goat x Sahelian sheep/goats) as well as Sahelian small ruminant genotypes. The eastern part of the country accounts for 60 % of the small ruminant population (Anonymous, 2000).

In order to further investigate the risk situation with respect to heartwater in the country, it was necessary to determine the distribution of *E. ruminantium* infection rates in the vector tick population. The study applied a molecular approach and started with the initial evaluation of three molecular PCR-based diagnostic tools, nested pCS20, nested *map1* and reverse line blot using nested PCR approach to determine *E. ruminantium* infection rates in *A. variegatum* ticks (**Chapter 3).** The nested PCR assay, based on pCS20 target sequences, showed the best performance and was considered most suitable for detection of *E. ruminantium* in *Amblyomma* ticks and useful tool for field epidemiological investigation of heartwater. The pCS20, *map1* and 16S PCRs have previously been used to detect *E. ruminantium* infection in ticks or animals (Allsopp et al., 1999; Kock et al., 1995; Mahan et al., 1998a; Peter et al., 2000, 1995; Simbi et al., 2003). This is the first time the three molecular assays, pCS20, *map1* and RLB, designed in a nested approach have been applied to vector populations, *A. variegatum* field ticks, to evaluate their comparative performance in detecting *E. ruminantium* infection. The reverse line blot has been applied before in the field (Oura et al., 2003) and the advantage of applying the assay to field-derived samples and in field epidemiological studies is the ability of the assay to detect multiple parasites and discover novel parasite species (Nijhof et al., 2003).

This study also demonstrated the first molecular evidence of *A. marginale*, *E. ovina*, and *A. ovis* infections in ticks in the study area in The Gambia thereby suggesting the presence of these parasites in the resident ruminant livestock populations. Clinically, it is impossible to differentiate fatal ovine ehrlichiosis from heartwater in areas where *Amblyomma variegatum* ticks are present

without microscopic examination (Scott, 1990). The presence of *E. ovina* in Keneba (the study area) cannot be discounted as significant number of heartwater-suspected mortalities among local dwarf sheep and dwarf goats could not be confirmed by microscopic examination of brain smears (Mattioli and Faburay unpublished data). Studies on *E. ruminantium* tick infection rates corroborated previous findings (**chapter 2**) of the existence of a gradient of heartwater risk in the country. Higher *E. ruminantium* infection rates were detected in the westerly part of the country, with prevalence ranging from 7.5 to 15 %, than in the easterly part, which showed prevalence range of 1.6 to 5.5 %. Thus a high degree of correlation between *E. ruminantium* seroprevalence and tick infection rates in the country further supports the conclusion that the success of future livestock upgrading programmes using more productive but highly susceptible to heartwater exotic genotypes of domestic ruminants could be jeopardized by the disease if adequate prevention and control measures are not put in place (Camus et al., 1996; Koney, 1995)

The epidemiology of heartwater in young animals, particularly under the traditional husbandry system, is not adequately understood. Several researchers postulated that the existence of endemic stability for *E. ruminantium* and tick-borne infections in general (O´Callaghan et al., 1998) may be dependent on infection, by tick transmission, of the very young host during a period of reduced susceptibility to clinical disease (Norval et al., 1992; Perry and Young, 1995). In a longitudinal study in extensively managed newborn lambs and kids, the onset (age at first infection) and kinetics of *E. ruminantium* infection was monitored using pCS20 PCR and MAP1-B ELISA (**Chapter 4).** This study showed that most neonatal lambs and kids appeared to carry maternal antibodies to *E. ruminantium* suggesting that they were born to dams that had previous exposure to *E. ruminantium* infection. *E. ruminantium*-specific DNA (pCS20) sequences were detected in some of the animals in the first three days of age, which apparently had no exposure to tick bites suggesting that vertical transmission of the organism from dam to offspring could be an occurrence. Although the mechanism, either *in utero* or by colostrum, is yet to be elucidated, vertical transmission of *E. ruminantium* has been demonstrated in calves under natural field conditions (Deem et al., 1996).

Many tick-borne pathogens related to *E. ruminantium*, such as *Anaplasma (Ehrlichia) phagocytophilum* (Wilson et al., 1964), *E. risticii* (Dawson et al., 1987), *Anaplasma* spp. (Zaugg, 1985) and *Coxiella burnetti* (Fiset et al., 1975) can be transmitted *in utero*. In a longitudinal study in Ghana, using PC-ELISA (Sumption et al., 2003) persistent antibodies to *E. ruminantium* were observed in calves throughout the study period and the possibility of vertical transmission was not ruled out (Bell-Sakyi et al., 2003). Thus it is concluded from this study that both the vector and vertical transmission may play a vital role in the epidemiology of heartwater in young animals. Survival analysis of the animals exposed to field challenge showed that the age range of 4 to 12 weeks corresponds to the period of highest susceptibility to heartwater in traditionally managed

small ruminants, and is suggested that vaccination in smallholder traditional systems should target this age range for maximum impact.

Pathogen diversity in host and vector population constitutes an important parameter of epidemiological investigation of infectious diseases. A study of the genetic diversity of *E. ruminantium* in ruminant hosts and *A. variegatum* tick vectors was carried out by analysis of the restriction fragment length polymorphisms (RFLP) of the gene encoding the major antigenic protein 1 (*map1*) (**Chapter 5).** Since ecological parameters could potentially influence the genetic evolution of pathogen populations, samples were collected from sites representative of the three main agroeceological zones, Sudano-Guinean (SG), Western Sudano-Sahelian (WSS), and Eastern Sudano-Sahelian (ESS), of The Gambia. At present there is no simple and reliable method for the molecular typing of different *E. ruminantium* stocks ((Jongejan and Bekker, 1999). In this study, the *map1* gene of *E. ruminantium* was considered an ideal target gene for genotypic characterisation (Allsopp et al., 1999) using PCR-RFLP (Geysen et al., 2003; Martinez et al., 2004) as it shows a high degree of sequence polymorphisms between isolates (Allsopp et al., 2001; Reddy et al., 1996), does not vary during host-tick passages (D. Martinez, unpublished) and is present in all *E. ruminantium* isolates from different geographic regions (Allsopp et al., 2001; Barbet et al., 1994; Van Vliet et al., 1994). The use of restriction endonuclease digestion of target genes (Brindley et al., 1993; Gasser et al., 1994; Geysen et al., 2003) or bacterial chromosomes (De Villiers et al., 2000), with infrequently cutting restriction endonucleases, has been applied to produce restriction profile fingerprints for strain- and species-specific identification. De Villiers et al. (2000) used *Sma*I and *Ksp*I restriction enzymes in separate digestions on whole genomic DNA and were unable to distinguish between the three West African isolates, Senegal (Jongejan et al., 1988), Sankat 430 (Bell-Sakyi et al., 1997) and Pokoase 417 (Bell-Sakyi et al., 1997). A possible reason for the lack of distinction between the *E. ruminantium* isolates was the digestion of whole genomic DNA instead of a specific amplified gene target, *map1*.

In the present study, *Alu*I restriction endonuclease, in a PCR-RFLP approach, was validated for 15 *E. ruminantium* reference strains and subsequently applied to field samples. Genetic clusters produced by the PCR-RFLP technique applied in this study showed high degree of agreement with the clusters of the *map1*-based phylogenetic analysis and in effect indicating the usefulness of the assay as a discriminatory tool for characterising genetic diversity of the pathogen in the field. The study showed the presence of at least 11 different *map1* genotypes of *E. ruminantium* in The Gambia with *map1* profiles identical to Kerr Seringe strain showing the highest distribution frequency throughout the country. The greatest diversity was observed at study sites in the Western Sudano-Sahelian and Sudano-Guinean zones. The former zone is host to a large Open-nucleus Ruminant Pure Breeding Programme characterised by regular introduction of breeding stock from diverse geographical areas, while the largest centre for trade in ruminant livestock in

the country is located in the latter zone, which encompasses the coastal area. These factors could contribute to the increased heterogeneity of the *E. ruminantium* population in these areas. It is thus demonstrated that multiple genotypes of *E. ruminantium* exist in the field and specifically in The Gambia, with the likelihood of a similar level of antigenic differences, which could constitute an obstacle to the development of a protective vaccine against heartwater.

Despite this, immunisation appears to be the most sustainable method to control the disease in traditional livestock husbandry systems in sub-Saharan Africa (Jongejan, 1991; Mahan et al., 2001; Martinez et al., 1994, 1996; Nyika et al., 2002; Pretorius et al., 2007; Vachiery et al., 2006; Zweygarth et al., 2005). Therefore, the efficacy of two vaccination methods, the inactivated (Gardel stock) vaccine and the attenuated (Senegal stock) vaccine, was evaluated in a controlled cross-protection vaccination experiment; and subsequently the attenuated vaccine under field conditions in the Gambia (C**hapter 6).**

The on-station experiment showed that the inactivated Gardel vaccine provided only partial protection against heterologous needle challenge with the local (Gambia) Kerr Seringe isolate showing a mortality rate of 57 % in the vaccinated sheep. This loss is quite significant in economic terms for resource-poor smallholder farmers in sub-Saharan Africa. Although an inactivated vaccine based on the Mbizi strain (Zimbabwe) showed improved protection against heterologous laboratory and field challenge (Mahan et al., 2001), the Gardel inactivated vaccine has so far only demonstrated improved protection against homologous needle challenge (Martinez et al., 1994, 1996). In West Africa, the performance of the vaccine in cross-protection experiments has been less satisfactory (Gueye et al. unpublished data). The failure of the Gardel inactivated vaccine to induce full cross-protection against challenge with Kerr Seringe was attributed to possible antigenic differences between the two stocks (Jongejan et al., 1988, 1993; Van Winkelhoff and Uilenberg, 1981). Considering the wide diversity of *E. ruminantium* stocks in the field it is highly unlikely that the inactived vaccine would perform better under field challenge; and therefore it was not further tested.

In the controlled attenuated vaccine experiment, the culture-derived attenuated Senegal (passage 72) isolate (Jongejan, 1991), demonstrated to be non-transmissible by ticks (Martinez, 1997), offered 100 per cent protection in sheep against needle challenge with the Kerr Seringe isolate. Sheep in the vaccinated group were inoculated with approximately 153,000 organisms of the attenuated *E. ruminantium* as determined by real-time PCR (Peixoto et al., 2005) and three sheep reacted transiently with elevated temperatures to the inoculum suggesting the need for further reduction in the amount of attenuated *Ehrlichia* used for immunisation. The protective efficacy of the attenuated Senegal vaccine was further evaluated in sheep exposed to natural field tick challenge. This time each sheep was immunized with approximately 23,000 organisms of the

attenuated inoculum. The vaccine induced a high level of protection, with 75 % survival rate among vaccinated sheep at the end of the study. Unlike the previous dose of the attenuated inoculum, the administered dose in the field trial did not provoke any apparent clinical reaction in the vaccinated sheep; this amount of *E. ruminantium* organisms falls within the estimated range of 3,000 to 500,000 attenuated *E. ruminantium* (Welgevonden) organisms shown to induce protective immune response in goats by (Zweygarth et al., 2005). This could be considered a reasonable estimate of the number of organisms of the attenuated *E. ruminantium* (Senegal stock) for inducing protective immune response against heartwater in sheep.

Importantly, restriction profile analysis of *map1* gene derived from brain samples of the study animals showed that nearly all control sheep died due to *E. ruminantium* whose profiles were highly similar to Kerr Seringe, whereas the vaccinated animals died from *E. ruminantium* of a different genotype. This appears to support the conclusion that the attenuated Senegal vaccine was fully cross protective against the local Kerr Seringe isolate.