Summary

Heartwater is an economically important infectious disease of ruminants caused by the rickettsia *Ehrlichia ruminantium*, and transmitted by ticks of the genus *Amblyomma*. Small ruminants are particularly susceptible and this thesis described results of the first systematic study of the epidemiology of the disease in The Gambia. Studies were carried out to determine the distribution of the disease in the country by assessing the prevalence in the host and vector population in The Gambia and further a study with the objective of improving understanding of the epidemiology of the disease in young small ruminants (lambs and kids). A study to elucidate the genetic diversity of the pathogen population across the country was carried out which provided information that would contribute to the development of a sustainable control measure or strategy for heartwater in the country; and finally, studies were carried out to find a sustainable control solution for the disease through vaccination of traditional smallholder Sahelian sheep.

Chapter 2 described the results of a point seroprevalence study using the MAP1-B enzyme-linked immunosorbent assay used to test 1,318 serum samples collected from sheep and goats in the five divisions of The Gambia to determine the *Ehrlichia ruminantium* seroprevalence rates and to assess the risk for heartwater. About half (51.6%) of the 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 the Western Division and 62.1% in the Lower River Division. Sheep in the two easterly divisions (Central River and Upper River divisions) showed the lowest seroprevalence of 29.3% and 32.4%, respectively, while those in the North Bank Division showed an intermediate prevalence of 40.6%. In goats, less than one-third (30.3%) of animals tested were positive. The highest seroprevalence was detected in goats in the 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 the Central River Division and 2.3% in the Upper River Division). The results show a gradient of increasing heartwater risk for susceptible livestock from the east to the west of The Gambia.

Three PCR-based diagnostic assays, a nested pCS20 PCR, nested *map*1 PCR and a nested reverse line blot (RLB) hybridization assay, were comparatively evaluated in an initial study to determine the performance of the assays to detect *E. ruminantium* in *A. variegatum* ticks. Subsequently the most suitable assay was applied to study the level and distribution of *E. ruminantium* infection rates in field ticks at different sites in The Gambia (**chapter 3**). The nested pCS20 PCR assay

showed the highest detection performance with a detection rate of 16.6 %; the nested *map*1 PCR showed a detection rate of 11 % and the RLB detected 6.2 %. The RLB, in addition, demonstrated molecular evidence of *Ehrlichia ovina, Anaplasma ovis* and *Anaplasma marginale* infections in The Gambia. Subsequent application of the pCS20 assay showed *E. ruminantium* tick infection rates in the country ranged from 1.6 % to 15.1 % with higher prevalences detected at sites in the westerly divisions (Western, Lower River and North Bank; range 8.3 % to 15.1%) than in the easterly divisions (Central River and Upper River; range 1.6 % to 7.5 %). This corroborated the findings reported in chapter 2 of the existence of a gradient in the distribution of heartwater disease risk for susceptible livestock in The Gambia which factor must be considered in the overall design of future upgrading programmes.

To improve our understanding of the epidemiology of heartwater in very young animals, a nested pCS20 PCR and MAP1-B ELISA were used in a longitudinal study to monitor the onset (age at first infection) and kinetics of *E. ruminantium* infection in newborn lambs and kids under a traditional husbandry system at three sites (Kerr Seringe, Keneba, Bansang) in The Gambia (**chapter 4**). The animals were monitored for field tick infestation and the comparative performance of the two assays in detecting *E. ruminantium* infection was also assessed. The infection rate detected by pCS20 PCR varied between 8.6 % and 54.8 % over the 162-day study period and the rate of infection increased with increasing age. Nineteen per cent of the animals in week 1 post-partum tested positive by pCS20 PCR with half of these infections (7/14) detected in the first 3 days after birth, suggesting that transmission other than by tick feeding had played a role. The earliest detectable *A. variegatum* infestation in the animals occurred in week 16 after birth. The prevalence of infection detected by MAP1-B ELISA varied, between 11.5 % and 90 %. In contrast, the serological assay detected the highest proportion of positive animals in week 1 with a gradual decline in seropositivity with increasing age. In addition, intermittent positivity by PCR was observed in study animals possibly due to fluctuating rickettsaemia. The results showed a low degree of agreement between the two assays. It was concluded that the use of pCS20 PCR supported by transmission studies and clinical data could provide more accurate information on heartwater epidemiology in endemic areas and single-occasion testing of an animal may not reveal its true infection status. The study supported the view that both the vector tick and vertical transmission may play a vital role in the epidemiology of heartwater in young animals; the age range of 4 and 12 weeks corresponds to the period of highest susceptibility to heartwater in traditionally managed small ruminants, which should be taken into consideration when intervention strategies are applied.

A further study was carried out (**chapter 5**) in two phases: i) evaluating the usefulness of the PCR-RFLP assay based on the *map1* coding sequence of *E. ruminantium* as a discriminatory tool to characterise genetic diversity, ii) applying the technique to small ruminants and field-derived

samples from *A. variegatum* ticks to characterise genotypic diversity of the organism in 3 main agroecological zones of The Gambia, Sudano-Guinean (SG), Western Sudano-Sahelian (WSS) and Eastern Sudano-Sahelian (ESS). Restriction enzyme analysis of *map1* gene of *E. ruminantium* from field samples (*A. variegatum* ticks and small ruminants) showed restriction fragment length polymorphisms (RFLP) among stocks. Analysis of different strains of *E. ruminantium* by PCR-RFLP showed a high degree of agreement between the technique and the *map1* sequence-based phylogenetic analysis indicating the usefulness of the technique for studying genetic diversity of the organism. The study showed the occurrence of mixed infections with *E. ruminantium* genotypes in ruminants and ticks. Restriction enzyme *map1* profile analysis indicated the presence in The Gambia of multiple genotypes (at least 11) of *E. ruminantium* with the greatest diversity detected in the WSS and SG zones. Profiles similar to the Kerr Seringe genotype showed the highest distribution frequency making the strain a suitable candidate for further characterisation in cross-protection studies. Three additional genotypes showed relatively high distribution frequency and are considered important for isolation and subsequent characterisation.

A final study was carried out in an attempt to develop a suitable vaccine for use in The Gambia or the subregion (**chapter 6**). A local stock of *E. ruminantium* (Kerr Seringe) was initially isolated and cultured *in vitro* for use as challenge material in vaccination trials using naive Sahelian sheep. An inactivated vaccine, prepared from *Ehrlichia ruminantium* (Gardel stock), and a live attenuated vaccine from *E. ruminantium* (Senegal stock), were evaluated in two independent on-station trials. A local stock of *E. ruminantium* (Kerr Seringe) was used as challenge material. Inactivated and live attenuated vaccines provided 43 % and 100% protection, respectively, against virulent needle challenge. In a subsequent field trial, the attenuated vaccine protected 75% of sheep against virulent tick challenge, which was fatal for all control sheep. Quantification by real-time PCR showed that an immunising dose of approximately 23,000 attenuated *E. ruminantium* organisms was sufficient. Moreover, restriction fragment length polymorphism (RFLP) analysis indicated that the local Kerr Seringe genotype caused mortality amongst control sheep, whereas fatalities in the vaccinated group could be attributed to a different genotype. The field trial was carried out in small area of Kerr Seringe in The Gambia over a 5-month observation period and should be repeated in other areas of the country. This would contribute to the further evaluation of the potential of attenuated vaccines to control heartwater, one of the most important rickettsial tick-borne diseases of small ruminants in West Africa.