Future directions of research

There are two major areas of focus for research that are relevant and important to control heartwater in traditional livestock husbandry system in sub-Saharan Africa:

i) development and application of improved diagnostics in surveys to study *E. ruminantium* infection rates in hosts and vectors (for disease risk mapping), and,
ii) the development of a protective vaccine against the disease that is suitable for use by resource-poor smallholder farmers in sub-Saharan Africa.

Vast areas in sub-Saharan Africa where *Amblyomma* vector ticks are prevalent and heartwater disease is endemic have not been surveyed to provide important baseline information on the risk of the disease in these areas. In West Africa, apart from relatively recent surveys of *E. ruminantium* infection in domestic ruminants carried out in Ghana ((Bell-Sakyi et al., 2004; Koney et al., 2004), Senegal (Gueye et al., 1993) and Gambia (**chapter 2**), the risk of heartwater in large areas in countries of Sierra Leone, Liberia, Guinea Bissao, Guinea Conakry and Nigeria, just to name a few, is not known because the prevalence of infection has not been studied. Although the need to improve the specificity of the MAP1-B ELISA due to cross-reactions with unidentified ehrlichial species (Kakono et al., 2003) remains, the diagnostic test, nonetheless, appeared suitable for risk mapping of heartwater in endemic areas in traditionally managed small ruminants as shown in the survey in The Gambia (**chapter 2**). Serological surveys should be complemented by studies, using molecular tools, of *E. ruminantium* infection rates in the vector population. These surveys will result in the identification and mapping of risk areas of hearwater thus facilitating the harmonisation of heartwater disease control policies among countries in the region. The net effect will be significant contribution to upgrading the regional livestock industry both in terms of enhanced regional trade and productivity.

Indigenous small ruminants, sheep and goats, in comparison to local cattle, are generally more susceptible to heartwater (Yunker, 1996), and therefore should be given particular attention in heartwater epidemiology. For instance in 2003, the International Trypanotolerance Centre in The Gambia, shipped indigenous sheep to Sierra Leone as part of the post-civil war restocking programme; nearly all animals died and heartwater was confirmed a possible cause as two of the specimen five brain samples examined at ITC were positive for *E. ruminantium* infection (B. Faburay, unpublished data). This highlights the need for investigation of the incidence of

heartwater and other tick-borne diseases in countries in this region and potential impact of heartwater on future development of the domestic livestock industry.

Significantly, in West Africa, the potential economic loss due to heartwater is not known because the prevalence and incidence of the disease across the region has not been investigated. Additionally, in an attempt to meet the growing demand for milk by the increasing urban population and to reduce reliance on imported milk, countries in the region are making efforts to develop peri-urban/urban dairy schemes using more productive exotic cattle genotypes. These animals are highly susceptible to heartwater and other tick-borne diseases (Koney, 1995) and findings from such surveys could provide proper guidance to governments or national authorities of countries in the region in making informed disease control policies and strategies such as vaccination.

An important aspect of heartwater epidemiology is the understanding of the genetic diversity of *E. ruminantium* in the field (Allsopp et al., 1999), which information could facilitate the study of antigenic diversity of the stocks in a particular locality. The *map1* gene is variable between stocks of *E. ruminantium* (Reddy et al., 1996) and appeared to be an ideal target for characterising genetic diversity of the pathogen in the field (Allsopp et al., 1999; Martinez et al., 2004)(**chapter 5**). However, as indicated in this thesis, it has the drawback of the inability to predict cross-protection between strains and therefore could not be used in cross-immunity studies. With the availability of whole genome sequences of *E. ruminantium* stocks, Welgevonden (Collins et al., 2005) and Gardel (Frutos et al., 2006), it may be possible to identify a suitable gene target(s) that could provide information on genetic diversity of the pathogen and predict cross-immunity between strains.

An area of heartwater epidemiology that has not been given adequate attention is the epidemiology of the disease in young animals. Specifically, the establishment of endemic stability in small ruminants under the extensive management system is not adequately understood. Several researchers postulated that the existence of endemic stability for *E. ruminantium* and tick-borne infections in general 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). This may not be the case for small ruminants considering the type of husbandry system in the traditional set-up in many countries of sub-Saharan Africa. Vertical transmission has been demonstrated to occur in calves under natural field conditions in Zimbabwe (Deem et al., 1996). It is reported in this thesis that both vertical transmission and transmission by ticks may play a role in the establishment and maintenance of endemic stability to heartwater in traditionally managed small ruminants. This will require further investigation as it has important implications on the targeting vaccinations, especially with respect to age, in field exposed small ruminants. In

addition, it has been confirmed in this thesis that one time point testing of a small ruminant by PCR or MAP1-B ELISA may not reveal its true infection status (Simbi et al., 2003). The concept of 'rickettsaemic wave' or fluctuating rickettsaemia needs to be further investigated and its potential significance for international or cross-border trade in livestock from heartwater endemic areas to heartwater-free regions.

The live attenuated Senegal stock fully cross-protected against heterologous needle challenge with the Kerr Seringe stock, and in a field trial it showed a high level of protection against field tick challenge. The field trial was carried out in a limited field area in Kerr Seringe in a 5-month observation period; and due to the reported wide genetic and antigenic differences among stocks of *E. ruminantium* in limited geographical areas in the field and should be repeated in other areas of The Gambia requiring a longer observation period. Attributes essential for a heartwater vaccine to be practical, acceptable and affordable throughout most of Africa, in countries where the infrastructure for vaccine delivery developed, should include: i) ease of administration; one shot, no-cold chain or follow-up required, ii) safety; no clinical reaction following immunization, and iii) efficacy; no clinical reactions following homologous or heterologous challenge and over 90 % of animals protected (Bell-Sakyi, 2004). These arguments seem to favour attenuated vaccines; however, the protective efficacy of the current attenuated vaccine, which was evaluated in a limited geographic location, was below this protective threshold. To develop an attenuated vaccine that could augment the level of protection by the current attenuated vaccine and also for possible use throughout The Gambia, it will be necessary to identify a local stock of *E. ruminantium* with a high frequency distribution in the country and ideally a broad spectrum of cross protection for possible inclusion in a cocktail vaccine. Characterization of genetic diversity of *E. ruminantium* in The Gambia (**chapter 5**) showed high frequency distribution of the Kerr Seringe stock and was considered a prime candidate for further cross-protection studies and possible attenuation.

Due to increasing cross-border and regional trade in livestock among ECOWAS (Economic Community of West African States) countries, the solution to developing an effective and sustainable control for heartwater using attenuated vaccines lies in a regional approach and future research should be planned and implemented in that direction. Successful attenuation of the Senegal (Jongejan, 1991), Gardel (Martinez, 1997), Welgevonden (Zweygarth and Josemans, 2001) and Plumtree (D. Mwangi, personal communication) stocks of *E. ruminantim*; and experiences with Sankat 430 (L. Bell-Sakyi, unpublished data), shows that i*n vitro* attenuation of additional *E. ruminantium* stocks is a possibility and should therefore be considered strategically within the framework of a regional heartwater vaccine

Moreover, the relative importance of other tick-borne haemoparasites for small ruminants, such as *Anaplasma ovis* and *Ehrlichia ovina* found at one of the study sites needs to be determined. Since studies reported here were restricted to small geographic locations in The Gambia, it will therefore be necessary to carry out broader studies to determine the actual prevalence of these infections in domestic ruminant populations in the country. Information derived from such studies should provide important insight into the potential impact these infections are likely to have on future livestock upgrading programmes using more productive but susceptible exotic ruminant livestock breeds and their crosses. Finally, concurrent infections, such as haemonchosis should also be taken into consideration when future heartwater vaccines are further evaluated under natural field conditions in the Gambia.

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