Chapter 1

Introduction and literature review

1.1 Introduction

Heartwater or cowdriosis is a tick-borne disease caused by an intracellular rickettsial pathogen previously known as *Cowdria ruminantium* but reclassified as *Ehrlichia ruminantium* (Dumler et al., 2001). The disease is transmitted by ticks of the genus *Amblyomma* and affects domestic ruminant species, cattle, sheep, goats and several wild ruminant species such as buffalo, giraffe, and antelope as well as some wild rodents (Lounsbury, 1900). Heartwater is usually an acute disease and may be fatal within days of the onset of clinical signs. The course of the disease varies from peracute, acute, sub-acute to mild, depending on age, immune status, breed and virulence of the strain (Uilenberg, 1983). As indicated by the name 'heartwater', a hydropericardium is a striking change in most animals that die of the disease (Henning, 1956).

The disease is a serious constraint to livestock improvement programmes throughout sub-Saharan Africa and through its occurrence on some islands in the Caribbean, poses a potential threat to ruminant species in mainland North, Central and South America. Uilenberg (1983) ranked heartwater second only to East Coast fever and tsetse transmitted trypanosomosis. However, in West Africa, where East Coast fever does not occur, it is arguably the most important tick-borne disease of ruminant livestock. At present, there is a lack of safe, practical and effective vaccine, and in endemic areas, the control of the disease relies on acaricides to prevent tick transmission and antibiotic treatment of clinical cases. There is lack of information on the epidemiology of the disease in most parts of Africa and studies into the incidence and prevalence of infection have until recently been hampered by the lack of sensitive and specific diagnostic tools that are particularly suitable for use in countries in Africa. Thus the focus of the current heartwater research network is on the search and development of improved diagnostic tools and vaccines.

1.2 *Ehrlichia ruminantium*

1.2.1 Classification

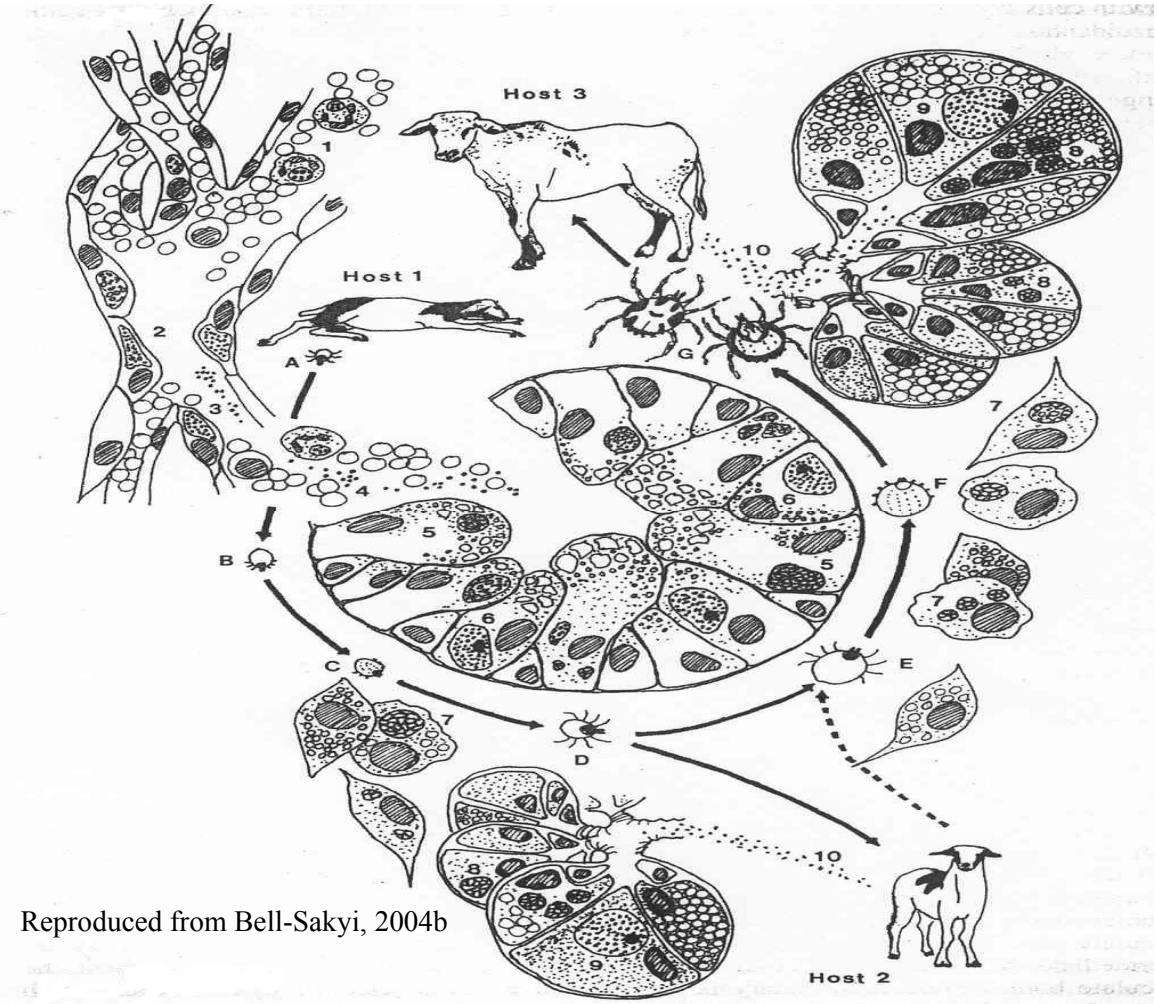
Ehrlichia ruminantium (Cowdry, 1925a) is an obligate intracellular rickettsial agent belonging to the family *Anaplasmataceae* (Dumler et al., 2001). The organism grows in membrane-bound vacuoles within the cytoplasm of the host cell (Kocan and Bezuidenhout, 1987; Pienaar, 1970; Prozesky et al., 1986). It infects mainly endothelial cells (Cowdry, 1926) and to a lesser extent neutrophils (Jongejan et al., 1989a; Logan et al., 1987). The heartwater organism is a member of the Class Proteobacteria and Order Rickettsiales. It was previously classified as a member of the family *Rickettsiaceae*, Tribe *Ehrlichieae* and Genus *Cowdria* (Moshkovski, 1947). The development of serodiagnostic tests revealed a very close antigenic relationship between *E. ruminantium* and the ehrlichial pathogens, *E. canis* and *E. chaffeensis* (Jongejan et al., 1993a; Katz et al., 1997; Kelly et al., 1994), and to a lesser degree, *Ehrlichia ovina* and *Ehrlichia bovis* (Jongejan et al., 1993a). Recent classification, based on genetic analyses of 16S rRNA genes,

groESL and surface protein genes, assigned the organism to the family *Anaplasmataceae* and to the genus *Ehrlichia* (Dumler et al., 2001).

1.2.2 Life cycle

Amblyomma spp. are the known vectors of *E. ruminantium* and are three-host ticks (Camus et al., 1996). The larvae and nymphs acquire infection by feeding on *E. ruminantium*-infected domestic or wild ruminants. Following acquisition feeding of *Amblyomma* larvae on *E. ruminantium*-infected hosts, rickettsiae were first seen by light microscopy occasionally in moulting larvae 27 days post detachment, and more frequently in the resultant unfed nymphs up to 101 days post detachment (Cowdry, 1925b). *E. ruminantium* were found in the midgut epithelial cells, and sometimes free in clumps in the gut lumen (Cowdry, 1925b) and also in the salivary gland (Hart et al., 1991; Kocan et al., 1987). Electron microscopy revealed *E. ruminantium* in the midgut of unfed nymphal or adult *A. hebraeum* and *A. variegatum*, previously fed on rickettsaemic hosts, and up to 4 days following initiation of transmission feeding (Kocan and Bezuidenhout, 1987). In addition, *E. ruminantium* organisms have also been detected in tick haemocytes and malpighian tubules (Du Plessis, 1985; Kocan and Bezuidenhout, 1987). The presence of the organism in a haemocyte after 2 days of transmission feeding (Hart et al., 1991) suggested a possible means of transfer between midgut and salivary gland (Bell-Sakyi, 2004b). Different morphological forms of the organism have been demonstrated in the salivary glands of feeding ticks suggesting the occurrence of a developmental cycle in the tick host. The presence of *E. ruminantium* in the midgut and salivary gland of the tick suggested that colonisation of both organs is necessary for the development cycle of the organism and that transmission of the organism by the tick to the vertebrate host occurs by either regurgitation of the gut content and/or through secretion of saliva while feeding. The period required for transmission of the organism to occur after attachment of an infected tick to a susceptible host is estimated between 27 and 38 hours for nymphs and between 51 and 75 hours for adults (Bezuidenhout, 1987). Infected animals serve as source of infection for ticks.

The ruminant host, once infected, may remain a carrier for up to 3.5 years (Andrew and Norval, 1989; Bekker et al., 2002b; Camus, 1992) and potentially for the rest of its life, thus serving as a reservoir of infection for ticks. Although there have been reports of transovarial transmission in *A. hebraeum* (Bezuidenhout and Jacobsz, 1986), ticks normally only transmit *E. ruminantium* intrastadially and therefore do not serve as a reservoir of infection in the absence of susceptible hosts. Vertical transmission in cattle of *E. ruminantium* from dam to calf has also been reported (Deem et al., 1996); but the mechanism as well as how frequent and widespread this phenomenon may be in ruminant species, cattle and small ruminants remain to be elucidated.



Reproduced from Bell-Sakyi, 2004b

Figure 1. *Life cycle of E. ruminantium (ER). Host 1 has been infected with ER. Morulae appear in circulating neutrophils several days later (1) and then subsequently in vascular endothelial cells (2). Elementary bodies (3) are released into the bloodstream. Amblyomma sp. larvae (A) ingest blood containing ER elementary bodies during feeding (4). During this period ER morulae can be seen in midgut epithelial cells (5 and 6) and sometimes in the lumen. After moult, Amblyomma nymphs (D) feed on host 2. After several days ER morulae are found in the salivary gland acinar cells (8 and 9) carried there from the midgut possibly by haemocytes (7). The feeding nymphs transmit ER to host 2 presumably as extracellular infective forms through their saliva (10). Host 2 becomes a source of infection for other immatures. The engorged nymphs (E) detach and moult (F) with ER morulae presumably still present in midgut endothelial cells (5 and 6). Following moult the Amblyomma adults feed on host 3; and after several days ER morulae are found in salivary gland acinar cells (8 and 9); carried there possibly by haemocytes (7). The feeding adults (G) transmit infective ER to host 3 in their saliva.*

However, a major mode of transmission of *E. ruminantium* to mammalian hosts occurs during feeding by an infected tick. Infective organisms are presumably first transported to regional lymph nodes draining the tick attachment site (Camus et al., 1996; Du Plessis, 1970). Several days after infection, rickettsiae were first detected in the cytoplasm of circulating neutrophils (Logan et al., 1987), although the numbers may vary depending on the *E. ruminantium* isolate (Jongejan et al., 1989b). This neutrophil stage almost coincides with the onset of clinical signs and the invasion of endothelial cells lining the blood vessels (Du Plessis, 1970). Jongejan et al. (1991a)

described the life cycle as a *Chlamydia*-like developmental cycle, which starts with the entry of an infectious stage, an electron dense elementary body, into the intracellular cytoplasmic vacuole of an endothelial cell. Elementary bodies divide by binary fission to produce large colonies of metabolically active reticulate bodies. Infected cells disrupt 5 to 6 days later resulting in the release of numerous elementary bodies in the bloodstream of host animals to begin a new cycle of infection. A schematic illustration and systematic explanation of the life cycle of the parasite is outlined in Figure 1.

1.2.3 *In vitro* cultivation

The first successful attempt to propagate *E. ruminantium* in arthropod cell lines was associated with the cultivation in primary cell cultures initiated from infected moulting nymphs of *A. hebraeum* and *A. variegatum* (Andreason, 1974). These cultures at nine days old caused fatal heartwater in sheep upon injection intravenously. Subsequent attempts ranged from failure (Uilenberg, 1983; Yunker et al., 1988) to success with the first continuous propagation of the Gardel stock of *E. ruminantium* in an *Ixodes scapularis* tick cell line for more than 500 days (Bell-Sakyi et al., 2000). Furthermore, a *Rhipicephalus appendiculatus* cell line, RAN/CTVM3, was infected with the Gardel stock of *E. ruminantium* (Bekker et al., 2002a) and continuous cell lines derived from *A. variegatum*, *I. scapularis* and *I. ricinus* were infected with *E. ruminantium* cultured in bovine endothelial cells (Bell-Sakyi, 2004a). Tick cell lines derived from six different tick species, *A. variegatum*, *B. decoloratus*, *B. microplus*, *I. scapularis*, *I. ricinus* and *R. appendiculatus*, were reportedly infected with *E. ruminantium* (Zweygarth, 2006).

The first relatively successful attempt to cultivate *E. ruminantium* in mammalian cells was the growth of primary kidney cell cultures from *E. ruminantium*-infected goats for which only cultures of 13 days old or less could induce hearwater in goats (Jongejan et al., 1980). However, *E. ruminantium* could not be detected microscopically in these cultures. Subsequently, a primary *E. ruminantium*-infected neutrophil culture, maintained *in vitro* for 18 h to 5 days, was established (Logan et al., 1987) and was successfully adapted for production of *E. ruminantium* antigen for heartwater serology (Jongejan et al., 1989a; Martinez et al., 1990). Continuous *in vitro* propagation of *E. ruminantium* in mammalian cells was first achieved using bovine umbilical cord endothelial cells (Bezuidenhout et al., 1985). This created new possibilities, and subsequent propagation of *E. ruminantium* used endothelial cells from various organs or anatomical sites of various ruminant or mammalian species. Endothelial cells derived from bovine aorta, bovine pulmonary artery, foetal bovine heart (Yunker et al., 1988), ovine pulmonary artery (Byrom et al., 1991), bovine saphenous vein (Neitz and Yunker, 1996) and caprine jugular vein were all successfully used. Additionally, endothelial cells derived from brain capillaries of bovine (Martinez et al., 1993b; Totté et al., 1996) and humans (Totté et al., 1993) supported the *in vitro* growth of *E. ruminantium*. Continuous *in vitro* propagation of *E. ruminantium* was also achieved in endothelial cells obtained from African wild ruminants (Smith et al., 1998) and non-ruminants

(Totté et al., 1993) such as sable antelope (*Hippotragus niger*), buffalo (*Syncerus caffer*), eland (*Tragelaphus oryx*) and from bush pig (*Potamochoerus porcus*). In addition, *E. ruminantium* was shown to grow in monocyte-macrophage cell lines from mice and dogs, and in human leukaemia cell line (HL-60) although with low rates of infection and without persistent infection (Zweygarth, 2006). The above results showed that growth of *E. ruminantium* is not restricted to cells derived from natural hosts of the organism and could potentially have an expanded host range. Importantly, its ability to grow in human endothelial cells adds to concern of the potential role as a human pathogen (Allsopp et al., 2005; Loftis et al., 2006).

In vitro cultivation of *E. ruminantium* enhanced the upscaling of mass production of the rickettsia for possible large scale vaccine production. An early attempt to bulk produce *E. ruminantium* was the propagation of endothelial cells, and subsequent infection with the organism in roller bottles of 800 cm^2 of culture area (Brett and Bezuidenhout, 1989). These researchers estimated that each bottle could yield 20,000 doses of live *E. ruminantium* vaccine. Subsequent studies on the adhesion properties of endothelial cells in a bioreactor showed that endothelial cells attached efficiently on collagen microspheres, although the experiment stopped short of infecting these cells with *E. ruminantium* (Totté et al., 1993). Recently, mass production of *E. ruminantium* elementary bodies using microcarriers as anchors for endothelial cells in stirred tank bioreactors was reported, opening the possibility of upscaling *E. ruminantium* production for vaccine production (Marcelino et al., 2006).

1.3 Heartwater

1.3.1 Background

The first description of a disease resembling heartwater or cowdriosis was made in South Africa (Trichardt, 1838) upon observation of a fatal nervous disease of sheep following massive tick infestation 3 weeks earlier (Neitz, 1968). Webb (1877) described the enormous losses amongst small ruminants due to heartwater, and believed that the disease was associated with the bont tick *A. hebraeum*. Dixon (1898) was able to transmit heartwater by the intravenous inoculation of infected blood into susceptible animals. It was then concluded that the disease was caused by a living microorganism and was thought to be a virus (Hutcheon, 1900). Lounsbury (1900) demonstrated that *A. hebraeum*, the major vector in southern Africa, was indeed the vector of heartwater. In 1925, Cowdry finally demonstrated the causative agent of heartwater in tissues of infected animals as well as in infected ticks, thereby describing for the first time a rickettsia causing a disease in domestic animals. Initially named *Rickettsia ruminantium*, the organism was later renamed *Cowdria ruminantium* in honour of Cowdry (Moshkovski, 1947) who successfully demonstrated gram-negative, intracellular coccus-like microorganisms in the tissues of heartwater-infected animals. *In vitro* culturing of the organism (Bezuidenhout et al., 1985) facilitated the development of diagnostic tests and the study of immunological responses in hosts to infection (Jongejan et al., 1991a; Martinez et al., 1993a; Totté et al., 1997), vaccines (Jongejan

et al., 1993b; Mahan et al., 1995; Mahan et al., 1994b, 1994; Totté et al., 1997). Extraction of genomic DNA allowed the development of PCR-based techniques for identification of pathogen DNA in ticks and in hosts, and to genetically characterise different isolates from geographically diverse areas (Kock et al., 1995; Mahan et al., 1992; Perez et al., 1997; Peter et al., 1995; Reddy et al., 1996; Waghela et al., 1991; Yunker et al., 1993).

1.3.2 Hosts

Domestic ruminants, notably cattle, sheep and goats are most susceptible to heartwater. A large number and variety of wild African and non-African ruminants are susceptible to infection with heartwater giving rise to the suspicion that some, in heartwater-endemic areas, may serve as reservoirs of the disease (Oberem and Bezuidenhout, 1987; Uilenberg, 1983). This suspicion was confirmed with the finding that the African buffalo (*Syncerus caffer*), which are excellent natural hosts for the vectors are, after recovery from heartwater, chronically infected and intermittently infective for ticks for many months (Andrew and Norval, 1989). (Peter et al., 2002) provided evidence that 12 African ruminants, three non-African ruminants and two rodents can become infected with *E. ruminantium*, in most cases asymptomatically. The wide host range of *E. ruminantium* is reflected in the ability to infect *in vitro* endothelial cells from a wide range of species including humans (Totté et al., 1993), African buffalo, warthog, giraffe, greater kudu, eland and sable antelope (Smith et al., 1998). Certain animals such as the crowned guinea fowl (*Numida meleagris*) and leopard tortoise (*Geochelone pardalis*) can serve as subclinical carriers of *E. ruminantium* and act as a source of organisms for ticks (Oberem and Bezuidenhout, 1987). Although the precise role of wildlife in the epidemiology of heartwater remains to be fully investigated, they transfer the infection to *Amblyomma* ticks, which feed on these hosts in nature (Oberem and Bezuidenhout, 1987). The involvement of wildlife in the cycle of heartwater, is a complicating factor for control of the disease and constitutes an important subject for further investigation (Uilenberg, 1983).

1.3.3 Vector ticks

One hundred and twenty nine *Amblyomma* species are known to exist on different continents (Jongejan and Uilenberg, 2004). However, vectors of heartwater in nature are all of African origin (Walker and Olwage, 1987). Members of this genus are widespread in tropical and sub-tropical zones where they have a wide host range, especially in immature stages (Jongejan and Uilenberg, 2004; Yunker, 1996). Sheep and goats are important hosts for immature stages of the two principal vectors, *A. hebraeum* and *A. variegatum*, the adults of which predominantly and preferentially infest cattle. *A. variegatum* is the most important species on most of the African continent and has the widest distribution throughout sub-Saharan Africa (Figure 2). It is the only African vector of heartwater that has established itself outside Africa, in the Caribbean region (Camus and Barre, 1987b). The distribution of this species covers most of sub-Saharan Africa

(Figure 2) as well as surrounding islands of Madagascar, La Reunion, Mauritius, Zanzibar, the Comoros and Sao Tomé (Uilenberg, 1983).

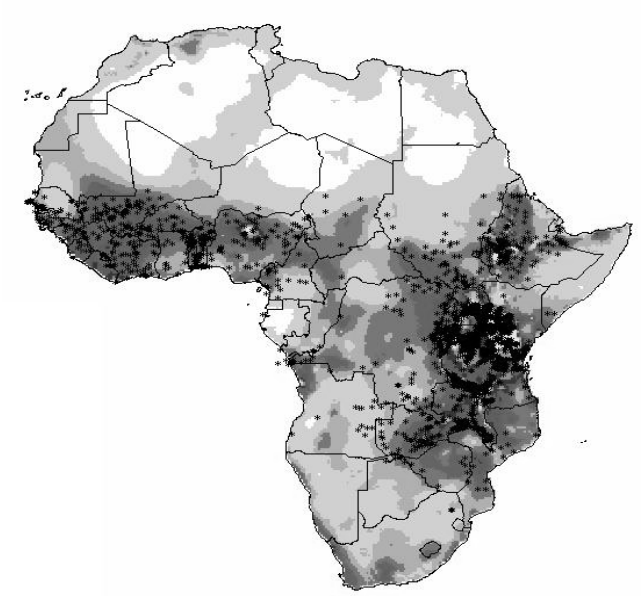


Fig. 2. Distribution of the main vector of Heartwater, *Amblyomma variegatum*, in Africa; Adopted from G.S. Cumming (1999)

A. hebraeum inhabits the southeastern part of Africa. Other species, *A. lepidum* and *A. gemma* inhabit parts of eastern and northeastern sub-Saharan Africa, *A. pomposum* occurring mainly in Angola, *A. cohaerens* and *A. astrion,* which have adapted from the African buffalo to livestock and found mainly in Ethiopia and São Tomé respectively, are of secondary importance in terms of vector capacity, distribution and frequency of parasitism of domestic livestock (Uilenberg and Niewold, 1981; Walker and Olwage, 1987). *A. marmoreum*, *A. sparsum* and *A. tholloni* also on the African continent, do not normally feed on domestic livestock (Peter et al., 2002; Uilenberg, 1983). In general, in Africa, the distribution of heartwater corresponds to that of the tick vectors (Uilenberg, 1983). On the American mainland, three potential, experimentally proven, vectors of *E. ruminantium* are known: *A. cajennense*, *A. maculatum* and *A. dissimile* (Jongejan, 1992; Uilenberg, 1982). *A. americanum*, *A. neumani* and *A. imitator* gave negative results in transmission experiments (Allan et al., 1998; Camus et al., 1996; Uilenberg, 1982). This shows the existence of a potential risk of introducing cowdriosis onto the American mainland. For instance, the establishment of *A. marmoreum* in Florida through importation of foreign wildlife has been reported (Allan et al., 1998), whereas *A. sparsum*, with some confirmed infected by PCR, was found on leopard tortoises imported from Zambia (Burridge et al., 2000). This threat is even more real in the face of the rapid increase in international trade and travel.

1.3.4 Clinical disease and immunity

Hearwater, in clinically affected animals, is characterised by sudden onset of high fever, which may be accompanied by nervous signs and may be followed more or less rapidly by death. The disease usually develops within 10 to 30 days after an infectious tick bite and the first symptom usually is a sudden rise in body temperature. Mortality rates vary between 5 % and 100 % (Camus et al., 1996; Van de Pypekamp and Prozesky, 1987). Mortality rate in indigenous goats in an endemic area of Guadeloupe has been estimated at around 10 % (Camus and Barre, 1987a). The disease occurs in susceptible ruminants following natural transmission by infected *Amblyomma* ticks, artificial transmission by inoculation of infected blood, tissue homogenate, ground-up tick suspension or ruminant endothelial cell culture (Camus et al., 1996). The severity of clinical signs and mortality rate depend on the species, breed and age of the ruminant host, the route of infection (tick transmitted or needle-inoculated), the virulence of the *E. ruminantium* isolate and the size of the inoculum. Young animals immediately after birth, irrespective of breed and the dam's immune status have been reported to possess innate resistance to heartwater (Uilenberg, 1983). The duration of this inverse-age resistance varies amongst species. It is reported to be 9 days in Merino lambs (Uilenberg, 1971), 2 weeks in kids (Camus and Barre, 1987a) and 2 to 3 weeks in calves (Uilenberg, 1983). Exotic breeds are more susceptible than indigenous livestock and mortality rate of 50 % or greater due to the disease have been reported in sheep and imported cattle in sub-Saharan Africa (Uilenberg, 1983). Merino sheep are highly susceptible (Alexander, 1931; Lounsbury, 1902), whereas goats of the Angora breed are the most susceptible of all domestic ruminants (Spreull, 1922). Generally, indigenous ruminant livestock in heartwater-endemic areas are resistant to the disease although this is less for sheep and goats. The course of clinical heartwater in ruminants varies. In the peracute form, death occurs suddenly with little or no prior indication of clinical disease. In the acute form, high fever of rapid onset is followed by anorexia, dyspnoea, nervous signs and death within 2-6 days. In the subacute form, the clinical signs are similar to those in the acute form, but less pronounced, and may be followed by death or recovery. In the mild or inapparent form, the only clinical sign is transitory fever, which may not be noticed in the field, followed by recovery and development of immunity. This form is common in neonatal animals, which possess an innate inverse age-related resistance to heartwater disease (Camus et al., 1996), and also in heartwater-endemic areas in disease-resistant indigenous breeds such as West African Dwarf goats and Djallonké sheep. As these symptoms are not pathognomonic, it is generally very difficult to diagnose heartwater in the live animal by clinical signs alone; fever is a feature of most infectious tick-borne and other diseases, and nervous signs may be symptomatic of poisoning, some nutritional disorders, or babesiosis and theileriosis, both of which have cerebral forms in cattle (Camus et al., 1996). Epidemiological factors such as presence or absence of the vector, *Amblyomma* spp. ticks including possible records of previous occurrence of heartwater in the locality should be considered in making a diagnosis.

Immunity to *E. ruminantium* infection (heartwater), similar to other intracellular parasites, is considered to be mainly cell-mediated (Totté et al., 1999, 1997). Antibodies produced in response to infection as early as the first day of fever (Viljoen et al., 1987), play little or no role in the protective immune response (Totté et al., 1999). Although serum antibodies are considered to engender no protective immunity, they appear to be a more useful indicator of exposure to the organism (Du Plessis, 1993). Animals that recovered from heartwater manifest solid immunity against homologous or antigenically related *E. ruminantium* isolate of several years' duration (Neitz, 1939). Cross-protection between isolates may be complete, partial or non-existent (Du Plessis et al., 1989; Jongejan et al., 1988b) and appears not to correlate with geographic origin. Antigenic diversity between *E. ruminantium* resulting in lack of protection between heterologous strains/stocks is the single most significant obstacle to developing a protective vaccine against heartwater and consequently constitutes a major constraint to livestock upgrading programmes in sub-Saharan Africa.

1.4 Epidemiology of Heartwater

1.4.1 Serodiagnosis

The indirect fluorescent antibody test (IFAT)(Du Plessis, 1981) was the first serological diagnostic assay used for large scale screening of *E. ruminantium* infection in heartwater epidemiology. Although this assay showed to be highly sensitive (Du Plessis and Malan, 1987), its specificity was affected by the detection of false-positive results, presumably due to a closely related *Ehrlichia* spp. in sera from *Amblyomma*-free areas (Du Plessis et al., 1987). Similarly, in a serological survey of *E. ruminantium* in cattle in Senegal (Gueye et al., 1993), interpretation of results was complicated by possible cross-reactions with *Anaplasma (Ehrlichia) bovis* known to occur in Senegal (Gueye et al., 1994b). Immunoblotting assays were also used in heartwater serology and cross-reactions with unknown *Ehrlichiae* were also noted (Jongejan et al., 1993a; Kobold et al., 1992; Mahan et al., 1993).

With the objective of improving the specificity of heartwater serology, an enzyme-linked immunosorbent assay (ELISA) was developed for *E. ruminantium* using infected *A. hebraeum* nymphs as antigen (Viljoen et al., 1987, 1985). However, *E. ruminantium* harvested from bovine endothelial cell cultures used as source of antigen in a competitive ELISA (Jongejan et al., 1991b) and two indirect ELISAs (Martinez et al., 1993a; Soldan et al., 1993) proved superior. The competitive ELISA based on the 32 kDa protein (Jongejan et al., 1991b) called major antigenic protein 1 (MAP1) was found to be conserved within the genus *Ehrlichia* (Jongejan et al., 1993a) and has been shown to cross-react with closely related *Ehrlichia* species, *Anaplasma* (*Ehrlichia*) *bovis* and *E. ovina*, (Jongejan et al., 1993a; Kobold et al., 1992; Mahan et al., 1993). *E. ruminantium* was also found to be closely related to *E. canis* (Jongejan et al., 1993a; Kelly et al., 1994). The recombinant major antigenic protein 1 (MAP1) was later introduced and an indirect ELISA based on a recombinant truncated form of the Major Antigenic Protein 1 of *E.*

ruminantium, (fragment B, thus named MAP1-B) has been developed (Van Vliet et al., 1995). This test does not cross-react with closely related *Ehrlichia* species found in ruminants, like *E. ovina* and *Anaplasma (Ehrlichia) bovis.* Although cross-reaction with *E. canis* (which infects dogs) and *E. chaffeensis* (a human pathogen), which do not infect ruminants in heartwater endemic regions, still exists, this cross-reactivity is not expected to hamper the results of heartwater serology. The MAP1-B ELISA (Van Vliet et al., 1995) has been used extensively by various laboratories where heartwater occurs (De Waal et al., 2000; Mahan et al., 1998; Mattioli et al., 2000a; Mondry et al., 1998; Peter et al., 2001; Semu et al., 2001). The test has been shown to perform satisfactory for small ruminants (De Waal et al., 2000; Mahan et al., 1998) with a specificity of 98.9 % and 99.4 % for caprine and ovine sera, respectively (Mondry et al., 1998; Van Vliet et al., 1995). It has been recommended that users of the assay in small ruminants in field situations should determine their own cutoff levels using local sera (Mboloi et al., 1999). The performance of MAP1-B ELISA has been less satisfactory with cattle exposed to continuous field challenge due to down regulation of MAP1-B antibody responses (Semu et al., 2001) resulting in detection of lower than expected seropositive rates in heartwater endemic areas (Mahan et al., 1998; Peter et al., 2001). Furthermore, a polyclonal competitive ELISA (PC-ELISA) for detection of antibodies to *E. ruminantium* has been described by Sumption et al. (2003). The assay was used in an earlier study prior to publication of the full method (Bell-Sakyi et al., 1996). Awa (1997) used a modification of the PC-ELISA in a survey of antibody prevalence in sheep and goats in north Cameroon. The PC-ELISA (Sumption et al., 2003) was comparatively evaluated using field sera from cattle and small ruminants (Bell-Sakyi et al., 2003); results indicated a better performance for the assay in comparison to MAP1-B ELISA with respect to detection of antibodies against *E. ruminantium* in cattle. In conclusion, serological assays, generally, unlike PCR-based techniques, only provide information about previous exposure of an animal to infection and do not differentiate between strains of *E. ruminantium*. Notwithstanding these limitations, serological tools still have important applications in heartwater epidemiology in sub-Saharan Africa, notably their use in disease risk mapping (Awa, 1997; Bell-Sakyi et al., 2004; Faburay et al., 2004; Koney et al., 2004). In addition, serological tests could be useful, when applied in conjunction with PCR, in determining the true infection status of animals in heartwater endemic regions prior to export to heartwater-free areas.

1.4.2 Molecular detection

Molecular cloning of several *E. ruminantium* genes resulted in the development of improved diagnostic tests for heartwater. The genes, *map2*, encoding the 21 kDa (Mahan et al., 1994a) and *map1* encoding the 32 kDa (Van Vliet et al., 1994) *E. ruminantium*-proteins have been cloned, characterized, sequenced and expressed to high levels to produce recombinant analogues, which have found application in subunit enzyme-linked immunosorbent assay (ELISA). The first reported attempt to identify and characterise *E. ruminantium* antigens was carried out using tissues harvested from the choroids plexus of goats, which died of heartwater (Jongejan and

Thielemans, 1989). A 32 kDa protein was detected in Western blotting by caprine and murine antisera raised against nine different *E. ruminantium* isolates. The murine antisera also recognised proteins of approximately 21 and 25 kDa (Jongejan and Thielemans, 1989). Subsequent immunoblotting analyses used antigen derived from *E. ruminantium*-infected endothelial cell cultures in which more proteins between the 21 and 32 kDa range were recognised by *E. ruminantium* antisera (Jongejan et al., 1993a, 1991b; Kobold et al., 1992; Lally et al., 1995; Mahan et al., 1994a, 1993; Perez et al., 1998; Roussow et al., 1990; Van Kleef et al., 1993).

Remarkable progress over the past decade in the development of polymerase chain-reaction (PCR)-based molecular diagnostics for *E. ruminantium* was associated with the development and use of DNA probes to detect *E. ruminantium* in *Amblyomma* ticks and in animals, with the subsequent significant increase in the sensitivity of these assays using PCR (Mahan, 1995). Waghela et al. (1991) first described the use of cloned DNA probes to detect the presence of *E. ruminantium* in *A. variegatum* ticks. One of the probes, the pCS20, showed high sensitivity and hybridized with all eight isolates of *E. ruminantium* tested and detected *E. ruminantium*-specific DNA from plasma samples from infected sheep before and during the febrile reaction (Mahan et al., 1992). Using PCR to amplify the *map1* gene, *E. ruminantium* could be detected in blood and bone marrow of sheep not only during the febrile response, but also up to 4 months later in animals, which recovered following treatment (Kock et al., 1995). In a comparative evaluation of detecting *E. ruminantium* infection, a DNA probe based on the pCS20 sequence also showed higher sensitivity and specificity relative to probes based on the 16S rDNA and *map1* gene sequences (Allsopp et al., 1999). The pCS20 PCR assay does not detect DNA of *E. canis* and *E. chaffeensis*, which, serologically and on the basis of the 16S rDNA analysis are closely related to *E. ruminantium* (Katz et al., 1997; Peter et al., 2000, 1995; Van Vliet et al., 1992). Furthermore, a recent technique, based on the16S rDNA, for simultaneous detection of a range of ruminant *Ehrlichia* and *Anaplasma* species by reverse line blot hybridisation detected *E. ruminantium* in experimentally-infected sheep during clinical response, but was not sufficiently sensitive to reliably detect the pathogen in animals shown to be carriers by xenodiagnosis (Bekker et al., 2002b).Against this background, assays based on the pCS20 sequences were considered the most sensitive and specific diagnostic test for *E. ruminantium* infection in ruminants and *A. variegatum* ticks (Peter et al., 2000; Simbi et al., 2003; Van Heerden et al., 2004b).

1.4.3 Molecular characterisation

Genetic characterization of *E. ruminantium* has been based on different gene targets, all of which manifest varying degree of nucleotide sequence polymorphisms among different isolates. The 16S rRNA gene was shown to manifest few nucleotide sequence differences in the hyper variable region (the V1 loop) and therefore considered most useful and often used for phylogenetic analysis (Allsopp and Allsopp, 2007; Rikihisa et al., 1997; Van Vliet et al., 1992). Genetic analyses of 16S rDNA, heat shock protein (groESL) and surface protein genes have reassigned the

heartwater agent to the genus of *Ehrlichia* (Dumler et al., 2001). In addition, the citrate synthase gene (*glt*A), which encodes the first enzyme of the tricarboxylic acid cycle, a key regulator of intracellular ATP production in virtually all living cells (Wiegarg and Remington, 1986), was shown to exhibit higher variation than the 16S rRNA gene and analysis of the gene sequence was considered one of the best tools for phylogenetic analysis and identification of *Ehrlichia* species (Inokuma et al., 2001). Compared to 16S sequences, *map1* gene sequences exhibited more polymorphisms (Reddy et al., 1996), and therefore considered a useful target to provide information about the distribution of *E. ruminantium* genotypes in the field (Allsopp et al., 1999; Martinez et al., 2004). Bekker et al. (2002a) reported differential transcription of *map1* gene paralog between ticks, bovine endothelial cell cultures and tick cell lines; and the gene has been confirmed to be one of a multigene family, with 16 tandemly arranged paralogs, which are all transcribed *in vitro* in infected bovine endothelial cells (Van Heerden et al., 2004a). Recent molecular characterisation of *E. ruminantium* using pCS20 sequences showed the usefulness of the sequences for phylogenetic analysis (Allsopp and Allsopp, 2007; Van Heerden et al., 2004b) Unlike *map1* gene sequences (Allsopp et al., 2001), phylogenetic analysis of pCS20 sequences of 14 *E. ruminantium* stocks showed distinct geographic clustering (with the exception of Kűmm1 isolate) of West and southern African isolates (Van Heerden et al., 2004b). The analysis showed no sequence variation amongst the West African isolates and indicated that the level of conservation of the *E. ruminantium* pCS20 region allows for reliable differentiation of the organism from other *Ehrlichia* spp. (Van Heerden et al., 2004b). Advances in genome sequencing resulted in the complete sequencing of the genome of two *E. ruminantium* isolates: the Welgevonden from South Africa (Collins et al., 2005) and Gardel from Guadeloupe (Frutos et al., 2006), which could impact positively on heartwater research notably through identification of possible novel vaccine targets.

1.4.4 Genetic diversity and recombination

Although *E. ruminantium* is shown to have no plasmids, phages, insertion sequences, or genes for pilus assembly (Collins et al., 2005) which mediate recombination in many bacteria (Allsopp and Allsopp, 2007), it possesses genes required for the assembly of a channel for bacterial competence (Collins et al., 2005) making it amenable to natural transformation (Thomas and Nielsen, 2005). This shows that the organism is capable of DNA uptake and homologous recombination by mechanisms which are currently not understood (Allsopp and Allsopp, 2007). On the other hand, intrachromosomal recombination events at repeated sequences that lead to deletions are considered to result in genome reduction (Rocha, 2003). Intracellular bacteria being unable to regain the lost sequences from other bacterial species through horizontal gene transfer thus suffer a loss of genes whose products must be obtained from the host (Collins et al., 2005). These deletions could result in the creation of new genotypes of *E. ruminantium* with possible phenotypic consequences. For example, the *map1-2* in the Gardel stock of *E. ruminantium* is shown to bear a deletion of 48 bp, which accounts for 80 % of the size difference between the

map1 clusters of the Gardel stock and the Welgevonden stock (Frutos et al., 2006). *map1-2* was shown to recombine with *map1-3* in a sub-strain, Gardel-CTVM, of the parent Gardel stock (Bekker et al., 2005). The deletion of *map1-2* gene in the CTVM Gardel subpopulation indicates that recombination can occur in *E. ruminantium*, which may influence the phenotypic characteristics of the bacteria (Bekker et al., 2005). These intrachromosomal recombination events could probably explain the wide genetic diversity observed amongst *E. ruminantium* stocks in the field. Futhermore, it has been postulated elsewhere that free-living bacteria readily acquire alien genes by horizontal gene transfer and this is considered a major mechanism in the evolution of bacterial pathogenesis (Ochman and Moran, 2001). The estimated proportion of genes acquired by this method of gene transfer by *E. ruminantium* is significantly low (approximately 3 %) (Collins et al., 2005) compared to that in other bacterial pathogens (of 10-15 %) (Merkl, 2004). This was attributed to the fact that only one species of intracellular parasite inhabits a host cell generally, which therefore restricts the parasite`s access to new genes (Andersson and Kurland, 1998). Thus for *E. ruminantium*, phenotypic change through intrachromosomal recombination seem a more frequent occurrence than changes resulting from homologous recombination. The latter phenomenon could occur between two genetically/antigenically different stocks by introduction into the genome of a new allelic variant of a gene or genomic region already present in that genome (described in Hughes and French, 2007). However, possible occurrence of extensive recombination between different stocks of *E. ruminantium* in the field has been reported in a recent study (Allsopp and Allsopp, 2007). The latter authors found that of the twelve *E. ruminantium* stocks from Southern, East and West Africa, only the Gardel stock did not show evidence of recombination. Extensive recombination was shown to have occurred among Southern and East African stocks, but not with West African stocks. The only evidence of recombination among the West African stocks was between Pokoase and Senegal sharing a *ftsZ* ortholog. The Kűmm 1, a Southern African stock, showed recombination with all the West African stocks (Allsopp and Allsopp, 2007).

Examination of synonymous substitution at individual loci revealed that both the original Welgevonden (South Africa) and the subvariant Welgevonden (maintained in Guadeloupe) included genes that showed evidence of homologous recombination with more distantly related genomes including genomes from the Gardel stock; such recombination was shown to be most evident in loci known to play roles in important biological processes (Hughes and French, 2007). Importantly, since homologous recombination results in both synonymous and non-synonymous changes, these recombination events could potentially have phenotypic effects on the recipient genotype (Hughes and French, 2007). It is postulated that the most likely time for recombination to occur is in the tick vector after ingestion of a blood meal from animal carrying a mixture of *E. ruminantium* genotypes and before the establishment of the organism in gut epithelial cells (Allsopp and Allsopp, 2007). The likelihood and frequency of homologous recombination occurring between different stocks of *E. ruminantium in vivo* in animals and ticks under natural

field conditions potentially resulting in creation of new genotypes of *E. rminantium* with different phenotypic characteristics should be investigated. Such information will have particular important implications on the implementation of live vaccination programmes to control heartwater in smallholder traditional livestock systems in sub-Saharan Africa.

1.4.5 *Ehrlichia*-like organisms and human cases

Recent reports suggest the existence of uncharacterised *Ehrlichia*-like organisms in heartwater-free areas. In the United States, a tick-borne *Ehrlichia* that is genetically and antignically similar to *E. ruminantium* was detected in *A. americanum* ticks collected from Panola Mountain Park, Atlanta, GA (Loftis et al., 2006). The *Ehrlichia* caused transient febrile illness in a goat reportedly similar to `heartwater fever` indicative of the mildest manifestation of *E. ruminantium* infecton (Camus et al., 1996; Jongejan et al., 1984). Preliminary genetic analysis based on 16S rRNA, *glt*A and *map*2 genes, as well as antigenic and biologic characterisations postulate that the agent is either a new species of *Ehrlichia* or a divergent strain of *E. ruminantium*. There is reported evidence that this agent might cause human disease (quoted in Loftis et al., 2006).

Similarly, a recent study described the detection by a PCR assay based on *E. ruminantium* pCS20 and 16S sequences in *Ehrlichia*-like organisms in animals and non-*Amblyomma* tick species in a heartwater-free area in South Africa (Northern Cape). Analysis of the sequences suggested the presence of a non-pathogenic variant of *E. ruminantium* in this area (Allsopp et al., 2007). Finally, recent evidence suggests that *E. ruminantium* might be infective for humans. Three fatal cases of suspected ehrlichiosis were diagnosed in three unrelated individuals who were not overtly immunocompromised. Two were children, both of whom died after a short illness of about a week, presenting a clinical picture of encephalitis with complaints of severe headache, sleepiness and an unsteady gait. The third individual died approximately 3 weeks after her dog died of `bilary fever`. Molecular evidence based on 16S rRNA and pCS20 gene sequences indicated that *E. ruminantium* was present in DNA from all 3 individuals (Allsopp et al., 2005).

1.5 Hearwater in The Gambia

1.5.1 Background

The Gambia is located in West Africa and is 400 km long and 30 km wide on both sides of the river Gambia (Figure 3). It stretches between 13°54´ and 13°20´ North and between 17°05´and 14°05´ West and borders in the west by the Atlantic ocean and by Senegal in the north, south and east. The country covers an area of 11, 300 km^2 with a total land area of 10,689 km^2. The

population is 1.4 million with an annual growth of 3.3 %. The population density is one of the highest in Africa with, on average, 138.8 persons/km^2.

The Gambia is situated in the Sudano-Sahelian agro-ecological zone. This zone varies from semi-arid (600-1000 mm per annum) in land to subhumid (more than 1000 mm per annum) at the coast. The humid and rainy season lasts from June to October with peak rainfall occurring in August. Temperature varies between 14-40 °C. The vegetation is mainly savannah woodland with swamp areas.

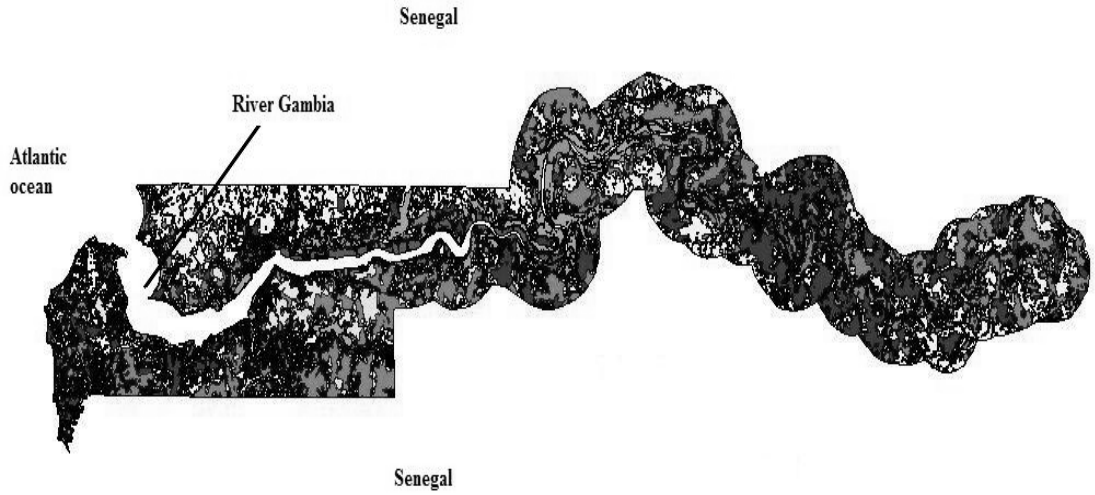


Figure 3. Map of The Gambia

The traditional farming system is either agropastoralism or small-scale mixed farming associated with rain-fed cultivation of cahs and food crops. The income derived from livestock and their products in such mixed farming systems is responsible for 10-50% of the household income (Wilson, 1991). Agriculture contributes about 25 % to Gross Domestic Product (GDP). Total agricultural land area is 7,140 km^2 and constitutes about 71.4 % of total landmass. Livestock accounts for about 25 % of Agricultural Gross Domestic Product with an annual growth rate of about 3.3 % reported in 1997 (FAO, 2005). This growth followed increased livestock integration into agriculture (Osaer and Goossens, 1999). Livestock production husbandry system is predominantly traditional especially in the rural countryside, with semi-intensive and intensification systems, in response to market demand, emerging in the peri-urban and urban areas.

1.5.2 Socio-economic importance

Heartwater or Cowdriosis is arguably the most important vector-borne disease in Africa affecting small ruminant productivity in smallholder agricultural systems, surpassing trypanosomosis; for which the natural prevalence in small ruminants is low (Murray et al., 1982). It causes an

estimated mortality rate of more than 10 % in indigenous dwarf sheep and goats and more than 50 % in ruminant livestock introduced from heartwater-free areas to endemic areas (Uilenberg, 1983). In Africa, the control of ticks and tick-borne diseases, among which heartwater disease is a key element, is considered the most important health and management problem, presenting a problem of equal or greater magnitude than tsetse fly and trypanosomosis (Young et al., 1988). Reliable figures for The Gambia and West Africa generally are unavailable, however, an annual national loss due to cowdriosis in Zimbabwe alone is estimated at US$5.6 million (Mukhebi et al., 1999), whereas other studies in cattle alone in large-scale commercial farms estimated it at 6.5 million US$ (Meltzer et al., 1996). The fact that in Sub-Saharan Africa smallholder livestock farmers predominate the livestock agricultural system, which is based on the traditional system of management where most causes of mortalities go unrecorded, the annual global loss for sub-Saharan Africa would be significantly high.

Nearly 80 % of all households in rural Gambia own a small ruminant, being a sheep or a goat (Sumberg, 1988). Improving small ruminant production and productivity is considered, therefore, a national priority to enhance the livelihood of resource-poor smallholder farmers and to ensure food and nutritional security for rural farming communities (UNDP/DLS, 1993). A participatory rural appraisal revealed that small-scale farmers raise small ruminants mainly to generate income, as savings, to obtain manure for their farm holdings and goat milk consumption (Bennison et al., 1997). In The Gambia, mixed farming or integrated crop-livestock production system predominate in smallholder systems (USAID, 1982). Thus any approach to develop agriculture on a sustainable basis with a view to alleviating poverty in smallholder farming communities, must consider a holistic approach of integrated livestock and crop production. A socio-economic study revealed that smallholder farmers in The Gambia regard keeping livestock as a more effective and quicker way of generating income and alleviating poverty than crop production, the performance of which over the years has been declining and unpredictable due to, among other factors, poor rains and unfavourable marketing conditions (DLS, 2002). In 2005, ITC introduced the Livestock Farmer Field School concept as an alternative and more participatory approach to identifying the specific constraints that small-scale livestock keepers encounter and to actively involve them in the decision-making process of choosing the appropriate technologies. Using a Participatory Epidemiology methodology, many small-scale farmers ranked heartwater second to PPR (a viral infection) in terms of its impact on the livelihood of farming communities (Hoeven et al., 2005). This underscores the importance of controlling heartwater in smallholder livestock agricultural systems in The Gambia and by extension in West Africa as a sustainable strategy of increasing the income and enhancing the well being of small-scale farmers.

Domestic ruminants of indigenous origin demonstrate variable degree of resistance to heartwater. Local breeds of cattle, N'Dama and Gobra zebu, appear more resistant to heartwater (Gueye et al., 1982), than local dwarf goats and sheep (Bell-Sakyi, 2004b). Higher rate of mortality due to

cowdriosis was reported among experimentally *Trypanosoma congolense*-infected indigenous Gobra zebu cattle (Mattioli et al., 1994) and was attributed to trypanosome-induced immunosuppression (Whitelaw et al., 1979) resulting in increased host susceptibility to other pathogens (Mattioli et al., 2000b). In sheep and goats, mortality due to heartwater appears to be comparatively more frequent and the disease is usually characterised by cases of sudden death, characteristic of acute forms of the disease (Yunker, 1996). Frequent cases of sudden deaths in which cowdriosis has been the prime suspect, have been observed in both indigenous and Sahelian (from northern Senegal and Mauritania) sheep and goats. In a pure breeding flock of traditionally managed Djallonke sheep and West African Dwarf goats at the International Trypanotolerance Centre (ITC) station in Keneba, 30 % (sheep and goats combined) of the brain smears examined post mortem were positive for *E. ruminantium* elementary bodies (Table 1), which samples were derived from animals that manifested symptoms of cowdriosis prior to death. Thirty six per cent (41/113) of the brain samples from sheep were positive for *E. ruminantium* elementary bodies and for goats 25 % (39/158) were positive (Table 1). Among the flock, other cases of mortality were attributed to pneumonia, coccidiosis, enteritis, severe mange, starvation and accidental death. Furthermore, cases of mortality attributed to cowdriosis have been reported in indigenous sheep and goats and their crosses (Djallonké/WAD x Sahelian sheep/goats) following translocation from the eastern part of the country to the western part towards the coast. These cases were attributed, among other factors, to possible antigenic differences between stocks of *E. ruminantium* (Jongejan et al., 1988b) in different localities in the country. Although heartwater-associated mortalities in small ruminants occur throughout the year (Osaer and Goossens, 1999), the frequency appears higher in the early dry season, which coincides with pick abundance of *A. variegatum* nymphs.

Table 1. Proportion of Giemsa-stained brain smears positive for *E. ruminantium* (ER) from Djallonké sheep and West African Dwarf goats in the ITC purebreeding flock in Keneba from 1997 to 1999

Animals species	Year	Period of year	Total No. of samples	No. of ER-positive samples	Others*	% *E. ruminantium* 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**

*Others – moratlity cases related to pneumonia, coccidiosis, enteritis, mange, starvation and accidents

1.5.3 Hosts and vector ticks

The small ruminant population of The Gambia totaled approximately 408 000 in 2002 (FAO, 2005). About 60 % of small ruminants are located in the Central and Upper River Divisions (Anonymous, 2000). Although most of the small ruminant population in the country consist of the local dwarf sheep and dwarf goats, the population of long-legged Sahelian breeds and their crosses with local breeds has been gradually increasing fueled primarily by increased farmer preference for them as breeding stock (Bennison et al., 1997). Small ruminants, generally, are more susceptible to heartwater (Yunker, 1996), although local dwarf goats and sheep seem to be fairly resistant compared to their Sahelian counterparts or their crosses (Osaer and Goossens, 1999). The Sahelian stock and their crosses are concentrated in the eastern part of the country predominantly towards the border with northern Senegal where they thrive relatively better possibly due to the low heartwater-disease risk in the area (Faburay et al., 2004). The cattle population totaled approximately 327 000 in 2002 and the population has been increasing for two decades (1980 – 2000) at an annual rate of 1.1 % (FAO, 2005). Most of the cattle population in The Gambia constitutes the N'Dama with increasing number of N'Dama and Gobra zebu crosses. The Gobra zebu cattle, due to their high susceptibility to tsetse-transmitted trypanosomosis, are more concentrated in the northern fringes of the country bordering Senegal where the risk of trypanosomosis is significantly lower. Indigenous cattle, N'Dama, Gobra zebu and their crosses, are fairly resistant to heartwater (Gueye et al., 1982). A small population of Holstein/Jersey x N'Dama crosses is found in the peri-urban areas towards the coast of the country being produced and distributed by the collaborative Gambia Government-ITC periurban continuous F1 dairy scheme. These crosses have shown high susceptibility to heartwater, with an estimated mortality rate of 30 %, in their first three months after birth (ITC Disease risk monitoring, unpublished).

Four genera of ixodid ticks have been recorded as parasitising domestic ruminants in The Gambia, *Amblyomma*, *Boophilus*, *Hyalomma and Rhipicephalus* (Claxton and Leperre, 1991; Mattioli et al., 1993; Morrel, 1958, 1964). In the subhumid zone of West Africa, generally, *A. variegatum* is the most abundant tick infesting cattle (Gueye et al., 1994b; Konstantinov et al., 1990; Vercruysse et al., 1982). In The Gambia, Simpson (1911) first described the presence of the ixodid tick, *A. variegatum,* the major vector of heartwater in West Africa. Peak abundance of the adult tick species in the country occurs in the rainy season, July to August (Claxton and Leperre, 1991; Mattioli et al., 2000a) and of the nymphs in the dry season, December to January (Mattioli et al., 2000a).

1.6 Other ticks and tick-borne diseases

The different tick species recorded on domestic ruminants in The Gambia include *A. variegatum*, *B. decoloratus*, *B. geigyi*, *H. truncatum*, *H. marginatum rufipes*, *R. senegalensis*, *R. evertsi evertsi*, *R. guilhoni*, *R. lunulatus* (Mattioli et al., 1994) and *R. mushamae* (B. Faburay , unpublished information). In The Gambia, the occurrence of tick-borne haemoparasites,

Anaplasma marginale, *Babesia bigemina* and *B. bovis* have been reported in indigenous N'Dama cattle (Kuttler et al., 1988; Murray et al., 1981). In a survey under traditionally managed N'Dama cattle at a study site in The Gambia, the overall prevalence of *A. marginale*, *B. bigemina* and *B. bovis* was 3.2 %, 0.9 % and 0.1 % respectively. Peak of *A. marginale* and *B. bigemina* microorganisms was reported to occur at the end of the rainy season towards the beginning of the dry season. Serological survey indicated prevalences: *A. marginale* (29.6 %), *B. bigemina* (44.7 %) and *B. bovis* (5.2 %)(Mattioli et al., 1997). Similar proportion of *B. bigemina* seroreactors was recorded in N'Dama cattle in Mali without any reported clinical cases (Miller et al., 1984). Seroprevalences of approximately 25 % for *A. marginale* and 50 % for *B. bigemina* in cattle over 1 year old, together with low occurrence of overt tick-borne diseases, were considered to be associated with a condition of endemic stability (Jongejan et al., 1988a). Similarly, the absence of clinical cases of anaplasmosis and babesiosis in N'Dama cattle in The Gambia suggests the establishment of endemic stability in the cattle population (Kuttler et al., 1988; Mattioli et al., 1997). The absence of positive cases of *A. marginale* in N'Dama cattle in The Gambia was attributed to the presence of a resistance factor towards this particular organism (Kuttler et al., 1988). However, other researchers reported the occurrence of anaplasmosis in Gambian N'Dama (Dwinger et al., 1989; Murray et al., 1981). Mattioli et al. (1997) postulated that the frequency of *A. marginale* is possibly affected by intercurrent trypanosomosis infections and that the level of trypanotolerance may be associated with a resistance factor towards *A. marginale per se*.
Evidence of the presence in The Gambia of a number of rickettsial pathogens in addition to *E. ruminantium* is provided in this thesis.

1.7 Control

1.7.1 Treatment and acaricides

Treatment of heartwater with sulfonamides or antibiotics was found to be effective at the onset of clinical symptoms and showed ineffective when defined nervous symptoms occurred (Camus et al., 1996; Zweygarth, 2006). In general, antibiotics of the tetracycline group are effective chemotherapeutic agents for successful treatment of heartwater. However, their use is constrained by the acute nature of the disease, which does not always allow timely intervention to prevent a fatal outcome; and in addition, they are expensive.

The application of acaricides by regular spraying or dipping of livestock is quite effective in preventing heartwater. However, the chemicals are expensive, and their use cause environmental and health concerns. The problem is further complicated by the ability of the ticks to develop acaricide resistance (Jongejan and Uilenberg, 2004). Furthermore, prevention of transmission results in animal populations, which are fully susceptible to heartwater and any other locally occurring tick-borne pathogens, and thus at risk of disease outbreaks if control measures break down (Norval, 1978).

1.7.2 Inactivated vaccines

The advent of *in vitro* tissue culture for *E. ruminantium* created the first opportunities of developing inactivated vaccines against heartwater. Inactivated elementary bodies mixed with Freund`s adjuvant were successfully used in immunization trials in small ruminants (Mahan et al., 1995; Martinez et al., 1993b, 1994, 1996) and cattle (Totté et al., 1997). Subsequent vaccination trials used more improved adjuvant formulations, Montanide ISA 50 or Quil A in goats (Martinez et al., 1996) or sheep (Mahan et al., 1998). In a field trial, an inactivated vaccine was used to immunize cattle, goats and sheep with significant reduction in mortality (Mahan et al., 2001). Although much safer than live vaccines, inactivated vaccines required several doses spread over a period of weeks or months during which the animals had to be kept tick-free (Bell-Sakyi, 2004b). The duration of immunity induced by inactivated vaccines is also limited, although persistence of antibodies and protection of up to 17 months in experimental goats has been reported (Martinez et al., 1996).

1.7.3 Attenuated vaccines

The first successful attenuation of *E. ruminantium* was achieved with the Senegal stock after 11 serial *in vitro* passages in bovine endothelial cell cultures. The attenuated stock although conferred protection against homologous challenge (Jongejan, 1991) failed to provide cross protection against heterologous needle challenge using stocks from East and Southern Africa (Jongejan et al., 1993b). On the other hand, the outcome of a related field trial in Senegal using attenuated Senegal isolate at passage 21 was complicated by concurrent infections, ehrlichiosis and anaplasmosis. Of the 30 vaccinated sheep, 13 animals died and *E. ruminantium* was detected only in two sheep, which had previously suffered from ehrlichiosis or anaplasmosis (Gueye et al., 1994a). In contrast to the Senegal stock, the Welgevonden stock did not attenuate after 17 culture passages (Jongejan, 1991). The latter stock attenuated upon passage in canine monocyte-macrophage cell line (Zweygarth and Josemans, 2001) and protected sheep against virulent needle challenge with four heterologous *E. ruminantium* stocks (Zweygarth et al., 2005). The Gardel stock became attenuated after 200 passages in culture (Martinez, 1997), whereas Crystal Springs stock, although lost its virulence only after higher passages (S.M. Mahan, personal communication), was not attenuated after 192 days in culture (Mahan et al., 1995). These results indicate that the capacity to attenuate is dependent on the stock of *E. ruminantium* and highlights the possibility of attenuating additional stocks of *E. ruminantium* from different geographic regions that may have broad spectrum of cross-protection. Furthermore, the attenuated Welgevonden stock was reported to be tick-transmissible (Zweygarth et al., 2005). In contrast, the attenuated Senegal and Gardel stocks were reported to be non-tick-transmissible (Martinez, 1997). Although transmissibility by ticks may restrict the use of a particular attenuated *E. ruminantium* stock outside its geographic origin for fear of reversion to virulence, it could prove beneficial since ticks infected with the attenuated organisms could transfer the vaccine to other naïve animals.

1.7.4 DNA vaccines

The potential of a DNA vaccine based on the *map1* gene to protect against heartwater has been examined in a mouse model with protection ranging from 23 % to 88 % (Nyika et al., 1998). The same vaccination method followed by protein boost augmented protection in mice against subsequent *E. ruminantium* challenge (Nyika et al., 2002). Cloned minilibraries of *E. rumnantium* in a *Salmonella* vaccine delivery system were used to immunise mice against heartwater with a survival rate of 14 % (Brayton et al., 1998). The polymorphic *cpg* gene from Welgevonden stock of *E. ruminantium* was examined for protection in mice and sheep with 80 % (4/5) protection against lethal challenge in the latter. None of the immunised mice were protected (Louw et al., 2002). A recombinant DNA vaccine consisting of four *E. ruminantium* open reading frames (ORF), known as the 1H12 ORFs, derived from the Welgevonden stock, provided protection against virulent homologous needle challenge in sheep in a cocktail formulation (Collins et al., 2003) or as individual ORFs (Pretorius et al., 2007). Prospects for the development of nucleic acid vaccines for heartwater appear promising. However, protection against virulent challenge has to date been achieved with homologous needle challenge. The critical step will be the evaluation of the protective efficacy of these prospective vaccines against tick challenge under field conditions.

1.8 Aims of the thesis

The aims of the studies described in this thesis were threefold:

- Firstly, a study was carried out to investigate and determine the importance of heartwater for susceptible livestock in The Gambia by assessing the prevalence of infection in host and vector population and mapping the level and distribution of heartwater-disease risk throughout the country,

- Secondly, it aimed to elucidate the genetic diversity of *E. ruminantium* in the country as a prerequisite to developing subsequent control strategies, and,

- Thirdly, to study the protective efficacies of available vaccines to control heartwater in traditional Sahalian sheep in the Gambia.

Chapter 2 describes results of a countrywide point seroprevalence study in field-exposed indigenous small ruminants using MAP1-B ELISA, considered to be the most highly specific and sensitive mass-screening diagnostic tool for ovine and caprine sera, to assess the level and distribution of heartwater disease-risk for susceptible livestock in The Gambia. Additional studies were carried out to further substantiate the above findings and present a complete picture of heartwater risk in the country.

Chapter 3 presents results of the comparison of three PCR-based molecular assays, nested pCS20 PCR, nested *map1* PCR and reverse line blot hybridisation, for detection of *E. ruminantium* in *A. variegatum* ticks and recommended the most suitable tool for application in heartwater epidemiology; the recommended diagnostic tool was subsequently applied to study the distribution of *E. ruminantium* infection rates in *A. variegatum* in The Gambia.

Chapter 4 presents findings of a 5-month longitudinal monitoring of *E. ruminantium* infection in newborn lambs and kids using pCS20 PCR and MAP1-B ELISA. The study monitored the kinetics (age at first infection) of *E. ruminantium* infection in the newborn animals with a view to improving our limited knowledge of the epidemiology of heartwater in young animals; in addition the comparative diagnostic performance of the two assays was compared.

Chapter 5 describes the results of a study, carried out in three major agroecological zones of The Gambia, aimed at understanding the genetic diversity of the heartwater agent by analysis of the various restriction fragment length polymorphisms of the gene encoding the major antigenic protein 1, *map1*, of *E. ruminantium* in the vector, *A. variegatum*, and the ruminant host.

To-date, there is no sustainable and cost-effective control measure developed against heartwater in susceptible livestock suitable for use in traditional livestock husbandry systems in The Gambia and sub-Saharan Africa generally. Efforts to find a solution through vaccination have been hampered by wide antigenic differences between *E. ruminantium* stocks resulting in lack of cross-protection between heterologous stocks.

Chapter 6 describes the results of an immunisation experiment in sheep using inactivated and attenuated *E. ruminantium* vaccines.

Chapter 7 provides a general discussion of results and proposed future research directions.

1.9 References

Alexander, R. A. (1931). Hearwater. The present state of our knowledge of the disease. 17th Report of the Director of Veterinary Services and Animal Industry, Union of South Africa, 89-149.

Allan, S. A., Simmons, L. A., and Burridge, M. J. (1998). Establishment of the tortoise tick *Amblyomma marmoreum* (Acari: *Ixodidae*) on reptile-breeding facility in Florida. J Med Entomol *35*, 621-624.

Allsopp, M. E. T. P., and Allsopp, B. A. (2007). Extensive genetic recombination occurs in the field between different genotypes of *Ehrlichia ruminantium*. Vet Microbiol, doi: 10.1016/j.vetmic.2007.1003.1012.

Allsopp, M. E. T. P., Hattingh, C. M., Vogel, S. W., and Allsopp, B. A. (1999). Evaluation of 16S, *map1* and pCS20 probes for the detection of *Cowdria* and *Ehrlichia* species. Epidemiol Infect *122*, 323-328.

Allsopp, M. E. T. P., Van Strijp, M. F., Farber, E., Josemans, A. I., and Allsop, B. A. (2007). *Ehrlichia ruminantium* variants which do not cause heartwater found in South Africa. Vet Microbiol *120*, 158-166.

Allsopp, M. T., Dorfling, C. M., Milliard, J. C., Bensaid, A., Haydon, D. T., Van Heerden, H., and Allsopp, B. A. (2001). *Ehrichia ruminantium* major antigenic protein gene (map1) variants are not geographically constrained and show no evidence of having evolved under positive selection pressure. J Clin Microbiol *39*, 4200-4203.

Allsopp, M. T., Louw, M., and Meyer, E. C. (2005). *Ehlichia ruminantium*-an emerging human pathogen. S Afr Med J *95*, 541.

Andersson, S. G., and Kurland, C. G. (1998). Reductive evolution of resident genomes. Trends Microbiol *6*, 263-268.

Andreason, M. P. (1974). Multiplication of *Cowdria ruminantium* in monolayer of tick cells. Acta Path Microbiol Scand Section B *82*, 455-456.

Andrew, H. R., and Norval, R. A. I. (1989). The career status of sheep, cattle and African buffalo recovered from heartwater. Vet Parasitol *34*, 261-266.

Anonymous (2000). Statistical yearbook of Gambian agriculture. 2000/2001 National Agricultural Sample Survey, Department of Planning, Department of State for Agriculture, The Republic of The Gambia, pp. 47.

Awa, D. N. (1997). Serological survey of heartwater relative to the distribution of the vector *Amblyomma variegatum* and other tick species in north Cameroon. Vet Parasitol *68*, 165-173.

Bekker, C. P. J., Bell-Sakyi, L., Paxton, E. A., Martinez, D., Bensaid, A., and Jongejan, F. (2002a). Transcriptional analysis of the major antigenic protein 1 multigene family of *Cowdria ruminantium*. Gene *285*, 193-201.

Bekker, C. P. J., De Vos, S., Taoufik, A., Sparagano, O. A. E., and Jongejan, F. (2002b). Simultaneous detection of *Anaplasma* and *Ehrlichia* species in ruminants and detection of *Ehrlichia ruminantium* in *Amblyomma variegatum* ticks by reverse line blot hybridization. Vet Microbiol *89*, 223-238.

Bekker, C. P. J., Postigo, M., Taoufik, A., Bell-Sakyi, L., Ferraz, C., Martinez, D., and Jongejan, F. (2005). Transcription analysis of the major antigenic protein 1 multigene family of three *in vitro*-cultured *Ehrlichia ruminantium* isolates. J Bacteriol *187*, 4782-4791.

Bell-Sakyi, L. (2004a). *Ehrlichia ruminantium* grows in cell lines from four ixodid tick genera. Path *130*, 285-293.

Bell-Sakyi, L. (2004b). Epidemiology of heartwater in Ghana and growth of *Ehrlichia ruminantium* in tick cell lines. PhD Thesis, Utrecht University, Utrecht, The Netherlands.

Bell-Sakyi, L., Koney, E. B. M., Dogbey, O., and K.J., S. (1996). Heartwater in Ghana: implications for control of ticks. Trop Anim Health Prod *28*, 59S-64S.

Bell-Sakyi, L., Koney, E. B. M., Dogbey, O., Sumption, K. J., Walker, A. R., Bath, A., and Jongejan, F. (2003). Detection by two enzyme-linked immunosorbent assays of antibodies to *Ehrlichia ruminantium* in field sera collected from sheep and cattle in Ghana. Clin Diagn Lab Immunol *10*, 917-925.

Bell-Sakyi, L., Koney, E. B. M., Dogbey, O., and Walker, A. R. (2004). *Ehrlichia ruminantium* seroprevalence in domestic ruminants in Ghana. I. Longitudinal survey in the Greater Accra Region. Vet Microbiol *100*, 175-188.

Bell-Sakyi, L., Paxton, E. A., Munderloh, U. G., and Sumption, K. J. (2000). Growth of *Cowdria ruminantium* the causative agent of heartwater, in a tick cell line. J Clin Microbiol *38*, 1238-1240.

Bennison, J. J., Barton, D., and Jaitner, J. (1997). The production objectives and feeding strategies of ruminant livestock owners in The Gambia: implications for policy makers. Agric Systems *55*, 425-444.

Bezuidenhout, J. D. (1987). Natural transmission of heartwater. Onderstepoort J Vet Res *54*, 349-351.

Bezuidenhout, J. D., and Jacobsz, C. J. (1986). Proof of transovarial transmission of *Cowdria ruminantium* by *Amblyomma variegatum*. Onderstepoort J Vet Res *53*, 31-34.

Bezuidenhout, J. D., Paterson, C. L., and Barnard, B. J. H. (1985). *In vitro* cultivation of *Cowdria ruminantium*. Onderstepoort J Vet Res *52*, 113-120.

Brayton, K. A., Van Der Walt, M., Vogel, S. W., and Allsopp, B. A. (1998). A partially protective clone from *Cowdria ruminantium* identified by using a *Salmonella* vaccine delivery system. Ann N Y Acad Sci *849*, 247-252.

Brett, M. S., and Bezuidenhout, J. D. (1989). Mass production of *Cowdria ruminantium* in tissue culture with the aim to replace the current heartwater blood vaccine with a tissue culture vaccine. Biennial Report of Veterinary Research Institute Onderstepoort, p46.

Burridge, M. J., Simmons, L. A., and Allan, S. A. (2000). Introduction of potential heartwater vectors and other exotic ticks into Florida on imported reptiles. J Parasitol *8*, 700-704.

Byrom, B., Yunker, C. E., Donovan, P. L., and Smith, G. E. (1991). *In vitro* isolation of *Cowdria ruminantium* from plasma of infected ruminants. Vet Microbiol *26*, 263-268.

Camus, E. (1992). Le portage asymptomatique de bovins et chevres Creole gueris de la cowdriose en Guadeloupe. Rev Elev Méd vét Pays trop *45*, 133-135.

Camus, E., and Barre, N. (1987a). Diagnosis of heartwater in the live animal: experiences with goats in Guadeloupe. Onderstepoort J Vet Res *54*, 291-294.

Camus, E., and Barre, N. (1987b). Epidemiology of heartwater in Guadeloupe and in the Caribbean. Onderstepoort J Vet Res *54*, 419-426.

Camus, E., Barre, N., Martinez, D., and Uilenberg, G. (1996). Heartwater (cowdriosis) a review. 2nd edition, O.I.E., Paris, 177pp.

Claxton, J. R., and Leperre, P. (1991). Parasite burdens and host susceptibility of Zebu and N'Dama cattle in village herds in The Gambia. Vet Parasitol *40*, 293-304.

Collins, N. E., Liebenberg, J., De Villiers, E. P., Brayton, K. A., Louw, E., Pretorious, A., Farber, F. E., Van Heerden, H., Josemans, A. I., Van Kleef, M., *et al.* (2005). The genome of the heartwater agent *Ehrlichia ruminantium* contains multiple tandem repeats of actively variable copy number. PNAS *102*, 838-843.

Collins, N. E., Pretorious, A., Van Kleef, M., Brayton, K. A., Allsopp, M. T., Zweygarth, E., and Allsopp, B. A. (2003). Development of improved attenuated and nucleic acid vaccines for heartwater. Dev Biol (Basel) *114*, 121-136.

Cowdry, E. V. (1925a). Studies on the aetiology of heartwater. I. Observation of a rickettsia, *Rickettsia ruminantium* (N. Sp.), in the tissues of infected animals. J Exp Med *42*, 231-252.

Cowdry, E. V. (1925b). Studies on the aetiology of heartwater. II. *Rickettsia ruminantium* (N. Sp.) in the tissues of ticks transmitting the disease. J Exp Med *42*, 253-274.

Cumming, G. S. (1999). The evolutionary ecology of African ticks. DPhil Thesis, University of Oxford, United Kingdom.

De Waal, D. T., Matthee, O., and Jongejan, F. (2000). Evaluation of the MAP1b ELISA for the diagnosis of heartwater in South Africa. Ann N Y Acad Sci *916*, 622-627.

Deem, S. L., Norval, R. A. I., Donachie, P. L., and Mahan, S. M. (1996). Demonstration of vertical transmission of *Cowdria ruminantium*, the causative agent of heartwater from cows to their calves. Vet Parasitol *61*, 119-132.

Dixon, R. W. (1898). Heartwater experiments. Agric J Cape of Good Hope *12*, 754-760.

DLS (2002). Livestock Development study in The Gambia: Detailed design and feasibility study. Final Report, July 2002.

Du Plessis, J. L. (1970). Pathogenesis of heartwater: I. *Cowdria ruminantium* in the lymph nodes of dometic ruminants. Onderstepoort J Vet Res *37*, 89-96.

Du Plessis, J. L. (1985). A method for determining the *Cowdria ruminantium* rate of *Amblyomma hebraeum*: effects in mice infected with tick homogenates. Onderstepoort J Vet Res *52*, 55-61.

Du Plessis, J. L. (1993). An *in vitro* test to demonstrate the inhibitory effect of homologous immune serum on the infectivity of *Cowdria ruminantium*. Onderstepoort J Vet Res *60*, 69-73.

Du Plessis, J. L., Camus, E., Oberem, P. T., and Malan, L. (1987). Heartwater serology: some problems with the interpretation of results. Onderstepoort J Vet Res *54*, 327-329.

Du Plessis, J. L., and Malan, L. (1987). The non-specific resistance of cattle to heartwater. Onderstepoort J Vet Res *54*, 333-336.

Du Plessis, J. L., Van Gas, L., Olivier, J. A., and Bezuidenhout, J. D. (1989). The heterogenicity of *Cowdria ruminantium* stocks: Cross immunity and serology in sheep and pathogenicity to mice. Onderstepoort J Vet Res *56*, 195-201.

Dumler, J. S., Barbet, A. F., Bekker, C. P. J., Dasch, G. A., Palmer, G. H., Ray, S. C., Rikihisa, Y., and Rurangirwa, F. R. (2001). Reorganisation of genera in the families *Rickettsiaceae* and *Anaplasmataceae* in the order *Rickettsiales*: unification of some species of *Ehrlichia* and *Ehrlichia* with *Neorickettsia*, description of six new species combinations and designation of *Ehrlichia equi* and 'HGE agent' as subjective synonymous of *Ehrlichia phagocytophila*. Int J Syst Evol Microbiol *51*, 2145-2165.

Dwinger, R. H., Agyemang, K., Grieve, A. S., Kora, S., and Jabang, B. (1989). Comparative study on N'Dama and Zebu cattle following experimental infection with *Trypanosoma congolense.* In: 20th International Scientific Council for Trypanosomiasis Research and Control, 10-14 April 1989, Mombasa, Kenya. Publication No. 115, pp. 394-401.

Faburay, B., Munstermann, S., Geysen, D., and Jongejan, F. (2004). A contribution to the epidemiology of *Ehrlichia ruminantium* infection (Heartwater) in small ruminants in The Gambia. Animal Health Research Working Paper No. 4, ITC (2004), pp.36.

FAO (2005). Livestock Sector Brief: The Gambia. Livestock Information, Sector Analysis and Policy Branch, March 2005, pp. 15.

Frutos, R., Viari, A., Ferraz, C., Morgat, A., Eychenie, S., Kandassamy, Y., Chantal, J., Bensaid, A., Coissac, E., Vachiery, N., *et al.* (2006). Comparative genomic analysis of three strains of *Ehrlichia ruminantium* reveals an active process of genome size plasticity. J Bacteriol *188*, 2533-2542.

Gueye, A., Jongejan, F., Mbengue, M., and Diouf, A. (1994a). Essai sur la terrain d'un vaccin attenue contre la cowdriose. Rev Elev Méd vét Pays trop *47*, 401-404.

Gueye, A., Martinez, D., Mbengue, M., Dieye, T., and Diouf, A. (1993). Epidemiologie de la cowdriose au Sénégal. II. Resultats de suivis sero-epidemiologiques. Rev Elev Méd vét Pays trop *46*, 449-454.

Gueye, A., Mbengue, M., and Diouf, A. (1994b). Ticks and haemoparasites of livestock in Sénégal. VI. The Soudano-Sahelian zone. Rev Elev Méd vét Pays trop *47*, 39-46.

Gueye, A., Mbengue, M., Kebbe, B., and Diouf, A. (1982). Note epizootologique sur la cowdriose bovine dans les Niayes au Sénégal. Rev Elev Méd vét Pays trop *35*, 217-219.

Hart, A., Kocan, K. M., Bezuidenhout, J. D., and Prozesky, L. (1991). Ultrastructural morphology of *Cowdria ruminantium* in mdgut epithelial cells of adult *Amblyomma hebraeum* female ticks. Onderstepoort J Vet Res *58*, 187-193.

Henning, M. W. (1956). Animal diseases in South Africa. 3rd ed. South Africa: Central News Agency Ltd.

Hoeven, E., Kora, S., and Manjang, A. (2005). Report on farmer field schools. In: ITC Annual Progress Report (2005), The Gambia.

Hughes, A. L., and French, J. Q. (2007). Homologous recombination and the pattern of nucleotide substitution in *Ehrlichia ruminantium*. Gene *387*, 31-37.

Hutcheon, D. (1900). History of heartwater. Agric J Cape of Good Hope *17*, 410-417.

Inokuma, H., Brouqui, P., Drancourt, M., and Raoult, D. (2001). Citrate synthase gene sequence: a new tool for phylogenetic analysis and identification of *Ehrlichia*. J Clin Microbiol *9*, 3031-3039.

Jongejan, F. (1991). Protective immunity to heartwater (*Cowdria ruminantium* infection) is acquired after vaccination with *in vitro* attenuated rickettsiae. Infect Immun *59*, 729-731.

Jongejan, F. (1992). Experimental transmission of *Cowdria ruminantium* (Rickettsiales) by the American reptile tick *Amblyomma dissimile* Koch, 1844. Exp Appl Acarol *15*, 117-121.

Jongejan, F., Bax, R., Meddens, M. J. M., and Quint, W. G. V. (1991a). *Cowdria* is recognized by a monoclonal antibody directed against the major outer membrane of *Chlamydia trachomatis*. Vet Microbiol *27*, 121-126.

Jongejan, F., De Vries, N., Nieuwenhuijs, J., Van Vliet, A. H. M., and Wassink, L. A. (1993a). The immunodominant 32-kilodalton protein of *Cowdria ruminantium* is conserved within the genus *Ehrlichia*. Rev Elev Méd vét Pays trop *46*, 145-152.

Jongejan, F., Morzaria, S. P., Omer, A. S., and Hashim, M. A. (1984). Isolation and transmission of heartwater (*Cowdria ruminantium* infection) in Blue Nile Province. Sud Vet Res Commun *8*, 141-145.

Jongejan, F., Perry, B. D., Moorhouse, P. D. S., Musisi, F. L., Pegram, R. G., and Snacken, M. (1988a). Epidemiology of bovine babesiosis and anaplasmosis in Zambia. Trop Anim Health Prod *20*, 234-242.

Jongejan, F., and Thielemans, M. J. C. (1989). Identification of an immunodominant antigenically conserved 32-kilodalton protein from *Cowdria ruminantium*. Infect Immun *57*, 3243-3246.

Jongejan, F., Thielemans, M. J. C., De Groot, M., Van Kooten, P. J., and Van Der Zeijst, B. A. M. (1991b). Competitive enzyme linked immunosorbent assay for heartwater using monoclonal antibodies to a *Cowdria ruminantium*-specific 32-kilodalton protein. Vet Microbiol *28*, 199-211.

Jongejan, F., and Uilenberg, G. (2004). The global importance of ticks. Parasitology *129*, S3-S14.

Jongejan, F., Uilenberg, G., Franssen, F. F. J., Gueye, A., and Nieuwenhuijs, J. (1988b). Antigenic differences between stocks of *Cowdria ruminantium*. Res Vet Sci *44*, 186-189.

Jongejan, F., Van Winkelhoff, A. J., and Uilenberg, G. (1980). *Cowdria ruminantium* (Rickettsiales) in primary goat kidney cell cultures. Res Vet Sci *29*, 392-393.

Jongejan, F., Vogel, S. W., Gueye, A., and Uilenberg, G. (1993b). Vaccination against heartwater using *in vitro* attenuated *Cowdria ruminantium* organisms. Rev Elev Méd vét Pays trop *46*, 223-227.

Jongejan, F., Wassink, L. A., Thielemans, M. J. C., Perie, N. M., and Uilenberg, G. (1989a). Serotypes in *Cowdria ruminantium* and their relationship with *Ehrlichia phagocytophilia*, determined by immunoflourescence. Vet Microbiol *21*, 31-40.

Jongejan, F., Wassink, L. A., Thielemans, M. J. C., Perie, N. M., and Uilenberg, G. (1989b). Serotypes in *Cowdria ruminantium* and their relationship with *Ehrlichia phagocytophilia*, determined by immunofluorescence. Vet Microbiol *21*, 31-40.

Katz, J. B., DeWald, R., Dawson, J. E., Camus, E., Martinez, D., and Mondry, R. (1997). Development and evaluation of a recombinant antigen, monoclonal antibody-based compitetive ELISA for heartwater serodiagnosis. J Vet Diagn Invest *9*, 130-135.

Kelly, P. J., Matthewman, L. A., Mahan, S. M., Semu, S., Peter, T., Mason, P. R., Brouqui, P., and Raoult, D. (1994). Serological evidence for antigenic relationships between *Ehrlichia canis* and *Cowdria ruminantium*. Res Vet Sci *56*, 170-174.

Kobold, A. M., Martinez, D., Camus, E., and Jongejan, F. (1992). Distribution of heartwater in the Caribbean determined on the basis of detection of antibodies to the conserved 32-kilodalton protein of *Cowdria ruminantium*. J Clin Microbiol *30*, 1870-1873.

Kocan, K. M., and Bezuidenhout, J. D. (1987). Morphology and development of *Cowdria ruminantium* in *Amblyomma* ticks. Onderstepoort J Vet Res *54*, 177-182.

Kocan, K. M., Bezuidenhout, J. D., and Hart, A. (1987). Ultrastructural features of *Cowdria ruminantium* in midgut epithelial cells and salivary glands of nymphal *Amblyomma hebraeum*. Onderstepoort J Vet Res *54*, 87-93.

Kock, N. D., Van Vliet, A. H. M., Charlton, K., and Jongejan, F. (1995). Detection of *Cowdria ruminantium* in blood and bone marrow samples from clinically normal, free-ranging Zimbabwean wild ungulates. J Clin Microbiol *33*, 2501-2504.

Koney, E. B. M., Dogbey, O., Walker, A. R., and Bell-Sakyi, L. (2004). *Ehrlichia ruminantium* seroprevalence in domestic ruminants in Ghana. II. Point prevalence survey. Vet Microbiol *103*, 183-193.

Konstantinov, O. K., Balde, M. C., Tchoumina, L. M., Mourzin, S. V., Popov, N. V., and Tchebotarev, A. N. (1990). Les tiques des la familles *Ixodidae* comme reservoir d'arbovirus en Republique de Guinee. I. Faune et ecologie des tiques. Rev Elev Méd Vét Pays Trop *43*, 85-95.

Kuttler, K. L., Clifford, D. J., and Touray, B. N. (1988). Prevalence of anaplasmosis and babesiosis in N'Dama cattle of The Gambia. Trop Anim Health Prod *20*, 37-41.

Lally, N. C., Nicoll, S., Paxton, E. A., Cary, C. M., and K.J., S. (1995). The *Cowdria ruminantium groE* operon. Microbiol *141*, 2091-2100.

Loftis, A. D., Will, R. K., Spurlock, J. P., Mahan, S. M., Danielle, T. R., Dasch, G. A., and Levin, M. L. (2006). Infection of a goat with a tick-transmitted *Ehrlichia* from Georgia, U.S.A., that is closely related to *Ehrlichia ruminantium*. J Vector Ecol *31*, 213-233.

Logan, L. L., Whyard, T. C., Quintero, J. C., and Mebus, C. A. (1987). The development of *Cowdria ruminantium* in neutrophils. Onderstepoort J Vet Res *54*, 197-204.

Lounsbury, C. P. (1900). Tick-heartwater experiment. Agric J Cape of Good Hope *16*, 682-687.

Lounsbury, C. P. (1902). Heartwater in sheep and goats. Agric J Cape of Good Hope *21*, 315-335.

Louw, E., Brayton, K. A., Collins, N. E., Pretorius, A., Van Strijp, F., and Allsopp, B. A. (2002). Sequencing of a 15-kb *Ehrlichia ruminantium* clone and evaluation of the *cpg* 1 open reading frame for protection against heartwater. Ann N Y Acad Sci *969*, 147-150.

Mahan, S. M. (1995). Review of the molecular biology of *Cowdria ruminantium*. Vet Parasitol *57*, 51-56.

Mahan, S. M., Andrew, H. R., and Tebele, N. (1995). Immunisation of sheep against heartwater with inactivated *Cowdria ruminantium*. Res Vet Sci *58*, 46-49.

Mahan, S. M., McGuire, T. C., Semu, S. M., Bowie, M. V., Jongejan, F., Rurangirwa, F. R., and Barbet, A. F. (1994a). Molecular cloning of a gene encoding the immunogenic 21 kDa protein of *Cowdria ruminantium*. Microbiol *140*, 2135-2142.

Mahan, S. M., Semu, S., Peter, T., and Jongejan, F. (1998). Evaluation of MAP1-B ELISA for cowdriosis with field sera from livestock in Zimbabwe. Ann N Y Acad Sci *849*, 259-261.

Mahan, S. M., Smith, G. E., and Byrom, B. (1994b). Conconavalin A-stimulated bovine T-cell supernatants inhibit growth of *Cowdria ruminantium* in bovine endothelial cells in vitro. Infect Immun *62*, 747-750.

Mahan, S. M., Tebele, N., Mukwedeya, D., Semu, S., Nyathi, C. B., Wassink, L. A., Kelly, P. J., Peter, T., and Barbet, A. F. (1993). An immunoblotting assay for heartwater based on the immunodominant 32-kilodalton protein of *Cowdria ruminantium* detects false positives in field sera. J Clin Microbiol *31*, 2729-2737.

Mahan, S. M., Waghela, S. D., McGuire, T. C., Rurangirwa, F. R., Wassink, L. A., and Barbet, A. F. (1992). A cloned DNA probe for *Cowdria ruminantium* hybridised with eight heartwater strains and detects infected sheep. J Clin Microbiol *30*, 981-986.

Marcelino, I., Sousa, M. F., Verissmo, C., Cunha, A. E., Carrondo, M. J. T., and Alves, M. P. (2006). Process development for mass production of *Ehrlichia ruminantium*. Vaccine *24*, 1716-1725.

Martinez, D. (1997). Analysis of the immune response of ruminants to *Cowdria ruminantium* infection. PhD thesis, Utrecht University, Utrecht, The Netherlands, 206pp.

Martinez, D., Coisne, S., Sheikboudou, C., and Jongejan, F. (1993a). Detection of antibodies to *Cowdria ruminantium* in the serum of domestic ruminants by indirect ELISA. Rev Elev Méd vét Pays trop *46*, 115-120.

Martinez, D., Maillard, J. C., Coisne, S., Sheikboudou, C., and Bensaid, A. (1994). Protection of goats against heartwater acquired by immunization with inactivated elementary bodies of *Cowdria ruminantium*. Vet Immun Immunopathol *41*, 153-163.

Martinez, D., Perez, J. M., Sheikboudou, C., Debus, A., and Bensaid, A. (1996). Comparative efficacy of Freund's and Montanide ISA50 adjuvants for the immunization of goats against heartwater with inactivated *Cowdria ruminantium*. Vet Parasitol *67*, 175-184.

Martinez, D., Sheikboudou, C., Couraud, P. O., and Bensaid, A. (1993b). *In vitro* infection of bovine brain endothelial cells by *Cowdria ruminantium*. Res Vet Sci *55*, 258-260.

Martinez, D., Swinkels, J., Camus, E., and Jongejan, F. (1990). Comparison de trois antigens pour le diagnostic de la cowdriose par immunofuorescence indirecte. Rev Elev Méd vét Pays trop *43*, 159-166.

Martinez, D., Vachiery, N., Starchurski, F., Kandassamy, Y., Raliniaina, M., Aprelon, R., and Gueye, A. (2004). Nested PCR for detection and genotyping of *Ehrlichia ruminantium:* use in genetic diversity analysis. Ann N Y Acad Sci *1026*, 106-113.

Mattioli, R. C., Bah, M., Faye, J. A., Kora, S., and Cassama, M. (1993). A comparison of field tick infestation on N⁾Dama, Gobra zebu and N⁾Dama x Gobra zebu cattle. Parasitology *47*, 139-148.

Mattioli, R. C., Bah, M., Reibel, R., and Jongejan, F. (2000a). *Cowdria ruminantium* antibodies in acaricide-treated and untreated cattle exposed to *Amblyomma variegatum* ticks in The Gambia. Exp Appl Acarol *24*, 957-969.

Mattioli, R. C., Faye, J. A., Bah, M., and Jabang, B. (1994). Experimental *Trypanosoma congolense* infection on naturally occurring ticks in N'Dama and Gobra zebu cattle. Parasitologia *36*, 305-311.

Mattioli, R. C., Janneh, L., Corr, N., Faye, J. A., Pandey, V. S., and Verhulst, A. (1997). Seasonal prevalence of ticks and tick transmitted haemoparasites in traditionally managed N'Dama cattle with reference to strategic tick control in The Gambia. Med Vet Entomol *11*, 342-348.

Mattioli, R. C., Pandey, V. S., Murray, M., and Fitzpatrick, J. L. (2000b). Immunogenetic influences on tick resistance in African cattle with particular reference to trypanotolerant N'Dama (*Bos taurus*) and trypanosusceptible Gobra zebu (*Bos indicus*) cattle. Acta Tropica *75*, 263-277.

Mboloi, M. M., Bekker, C. P. J., Kruitwagen, C., Greiner, M., and Jongejan, F. (1999). Validation of the indirect MAP1-B enzyme-linked immunosorbent assay for diagnosis of experimental *Cowdria ruminantium* infection in small ruminants. Clin Diagn Lab Immunol *6*, 66-72.

Meltzer, M. I., Perry, B. D., and Donachie, P. L. (1996). Mortality percentages related to heartwater and the economic impact of heartwater disease on large-scale commercial farms in Zimbabwe. Prev Vet Med *26*, 187-199.

Merkl, R. (2004). SIGI: score-based identification of genome islands. BMC Bioinformatics *5*, 22.

Miller, D. K., Diall, O., Craig, T. M., and Wagner, G. G. (1984). Serological prevalence of bovine babesiosis in Mali. Trop Anim Health Prod *16*, 71-77.

Mondry, R., Martinez, D., Camus, E., Liebisch, A., Katz, J. B., Dewald, R., Van Vliet, A. H. M., and Jongejan, F. (1998). Validation and comparison of three enzyme-linked immunosorbent assay for the detection of antibodies to *Cowdria ruminantium* infection. Ann N Y Acad Sci *846*, 262-272.

Morrel, P. C. (1958). Les tiques des animaux domestiques de l'Afrique occidentale francaise. Rev Elev Méd Vét Pays Trop *11*, 153-189.

Morrel, P. C. (1964). Distribution des *Rhipicephalus* du betail dans les steppes et savanes d'Afrique occidentale. Rev Elev Méd Vét Pays Trop *17*, 581-585.

Moshkovski, D. (1947). Comments by readers. Science *106*, 62.

Mukhebi, A. W., Chamboko, T., O´Callaghan, C. J., Peter, T. F., Kruska, R. L., Medley, G. F., Mahan, S. M., and Perry, B. D. (1999). An assessment of the economic impact of heartwater (*Cowdria ruminantium* infection) and its control in Zimbabwe. Prev Vet Med *39*, 173-189.

Murray, M., Clifford, D. J., Gettingby, G., Snow, W. F., and McIntyre, W. I. M. (1981). Susceptibility to African trypanosomiasis of N'Dama and zebu cattle in an area of *Glossina morsitans submorsitans* challenge. Vet Rec *109*, 503-510.

Neitz, A. H. W., and Yunker, C. E. (1996). Amino acid and protein depletion of in medium of cell cultures infected with *Cowdria ruminantium*. Ann N Y Acad Sci *791*, 24-34.

Neitz, W. O. (1939). The immunity in heartwater. Onderstepoort J Vet Sci Anim Industr *13*, 245-281.

Neitz, W. O. (1968). Heartwater. Bull Off Int Epiz *70*, 329-336.

Norval, R. A. I. (1978). The effects of partial beakdown of dipping in African areas in Rhodesia. Rhod Vet J *9*, 9-6.

Nyika, A., Barbet, A. F., M.J., B., and Mahan, S. M. (2002). DNA vaccination with *map1* gene followed by protein boost augments protection against challenge with *Cowdria ruminantium*, the agent of heartwater. Vaccine *20*, 1215-1225.

Nyika, A., Mahan, S. M., M.J., B., McGuire, T. C., Rurangirwa, F. R., and Barbet, A. F. (1998). A DNA vaccine protects mice against the rickettsial agent *Cowdria ruminantium*. Parasite Immunol *20*, 111-119.

Oberem, P. T., and Bezuidenhout, J. D. (1987). Heartwater in hosts other than domestic ruminants. Onderstepoort J Vet Res *54*, 271-275.

Ochman, H., and Moran, N. A. (2001). Genes lost and genes found: evolution of bacterial pathogenesis and symbiosis. Science *292*, 1096-1099.

Osaer, S., and Goossens, B. (1999). Trypanotolerance in Djallonke sheep and West African Dwarf goats in The Gambia: Importance of trypanosomosis, nutrition, helminth infections and management factors. PhD Thesis, Utrecht University, Utrecht, The Netherlands.

Perez, J. M., Martinez, D., Debus, A., Sheikboudou, C., and Bensaid, A. (1997). Detection of genomic polymorphisms among isolates of the intracellular bacterium *Cowdria ruminantium* by random amplified polymorphic DNA and southern blotting. FEMS Microbiology Letters *1*, 73-79.

Perez, J. M., Martinez, D., Sheikboudou, C., Jongejan, F., and Bensaid, A. (1998). Characterization of variable immunodominant antigens of *Cowdria ruminantium* by ELISA and immunoblots. Parasite Immunol *20*, 613-622.

Peter, T. F., Barbet, A. F., Alleman, A. R., Simbi, B. H., Burridge, M. J., and Mahan, S. M. (2000). Detection of the agent of heartwater, *Cowdria ruminantium*, in *Amblyomma* ticks by PCR: Validation and Application of the assay to field ticks. J Clin Microbiol *38*, 1539-1544.

Peter, T. F., Deem, S. L., Barbet, A. F., Norval, R. A. I., Simbi, B. H., Kelly, P. J., and Mahan, S. M. (1995). Development and evaluation of PCR assay for detection of low levels of *Cowdria ruminantium* infection in *Amblyomma* ticks not detected by DNA probe. J Clin Microbiol *33*, 166-172.

Peter, T. F., M.J., B., and Mahan, S. M. (2002). *Ehrlichia ruminantium* infection (heartwater) in wild animals. Trend Parasitol *18*, 214-218.

Peter, T. F., O´Callaghan, C. J., Medley, G. F., Perry, B. D., Semu, S. M., and Mahan, S. M. (2001). Population-based evaluation of the *Ehrlichia ruminantium* MAP-1B indirect ELISA. Exp Appl Acarol *25*, 881-897.

Pienaar, J. G. (1970). Electron microscopy of *Cowdria* (*Rickettsia*) *ruminantium* (Cowdry, 1926) in the endothelial cells of the vertebrate host. Onderstepoort J Vet Res *37*, 67-78.

Pretorius, A., Collins, N. E., Steyn, H. C., Van Strijp, F., Van Kleef, M., and Allsopp, B. A. (2007). Protection against heartwater by DNA immunisation with four *Ehrlichia ruminantium* open reading frames. Vaccine *25*, 2316-2324.

Prozesky, L., Bezuidenhout, J. D., and Paterson, C. L. (1986). Heartwater. an *in vitro* study of the ultrastructure of *Cowdria ruminantium*. Onderstepoort J Vet Res *53*, 153-159.

Reddy, G. R., Sulsona, C. R., Harrison, R. H., Mahan, S. M., Burridge M.J., and Barbet, A. F. (1996). Sequence heterogeneity of the major antigenic protein 1 genes from *Cowdria ruminantium* isolates from different geographical areas. Clin Diagn Lab Immunol *154*, 73-79.

Rikihisa, Y., Kawahara, M., Wen, B., Kociba, G., Fuerst, P., Kawamori, F., Suto, C., Shibata, S., and Futohashi, S. (1997). Western immunoblot analysis of *Haemobartonella muris* and comparison of 16S rRNA gene sequences of *H. muris*, *H. felis*, and *Eperythrozoon suis*. J Clin Microbiol *35*, 823-829.

Rocha, E. P. (2003). An appraisal of the potential for illegitimate recombination in bacterial genomes and its consequences: from duplications to genome reduction. Genome Res *13*, 1123-1132.

Roussow, M., Neitz, A. H. W., De Waal, D. T., Du Plessis, J. L., Van Gas, L., and Brett, M. S. (1990). Identification of the antigenic proteins of *Cowdria ruminantium*. Onderstepoort J Vet Res *57*, 215-221.

Semu, S. M., Peter, T. F., Mukwedeya, D., Barbet, A. F., Jongejan, F., and Mahan, S. M. (2001). Antibody responses to MAP 1B and other *Cowdria ruminantium* antigens are down regulated in cattle challenged with tick-transmitted heartwater. Clin Diagn Lab Immunol *8*, 388-396.

Simbi, B. H., Peter, T. F., Burridge, M. J., and Mahan, S. M. (2003). Comparing the detection of exposure to *Ehrlichia ruminantium* infection on a heartwater-endemic farm by the pCS20 polymerase chain reaction and an indirect MAP1-B enzyme linked immunosorbent assay. Onderstepoort J Vet Res *70*, 231-235.

Simpson, J. J. (1911). Entomological research in British West Africa. I. Gambia. Bull Entomol Res *2*, 187-239.

Smith, G. E., Anderson, E. C., M.J., B., Peter, T. F., and Mahan, S. M. (1998). Growth of *Cowdria ruminantium* in tissue culture endothelial cell lines from wild African mammals. J Wildlife Dis *34*, 297-304.

Soldan, A. W., Norman, T. L., Masaka, S., Paxton, E. A., Edelsten, M., and K.J., S. (1993). Seroconversion to *Cowdria ruminantium* of Malawi zebu calves, reared under different tick control strategies. Rev Elev Méd Vét Pays Trop *46*, 171-177.

Spreull, J. (1922). Heartwater. J. Dept. of Agric., Union of South Africa. *4*, 236-245.

Sumberg, J. (1988). Notes on cattle, sheep and goats. Department of Animal Health and Production, Abuko, The Gambia, pp.12.

Sumption, K. J., Paxton, E. A., and Bell-Sakyi, L. (2003). Development of a polyclonal competitive enzyme-linked immunosorbent assay for detection of antibodies to *Ehrlichia ruminantium*. Clin Diagn Lab Immunol *10*, 910-916.

Thomas, C. M., and Nielsen, K. M. (2005). Mechanisms of, and barriers to, horizontal gene transfer between bacteria. Nat Rev Microbiol *3*, 711-721.

Totté, P., Bensaid, A., Mahan, S. M., Martinez, D., and McKeever, D. J. (1999). Immune response to *Cowdria ruminantium* infections. Parasitology Today *15*, 286-290.

Totté, P., Blankaert, D., Marique, T., Kirkpatrick, C., Van Vooren, J. P., and Werenne, J. (1993). Bovine and human endothelial cell growth on collagen microspheres and their infection with the rickettsia *Cowdria ruminantium*: prospects for cells and vaccine production. Rev Elev Méd Vét Pays Trop *46*, 153-156.

Totté, P., McKeever, D., Martinez, D., and Bensaid, A. (1997). Analysis of T-cell responses in cattle immunized against heartwater by vaccination with killed killed elementary bodies of *Cowdria ruminantium*. Infect Immun *65*, 236-241.

Totté, P., Vachiery, N., Martinez, D., Trap, I., Ballingall, K. T., MacHugh, N. D., Bensaid, A., and Werenne, J. (1996). Recombinant bovine inteferon gamma inhibits the growth of *Cowdria ruminantium* but fails to induce major histocompatibility class II following infection of endothelial cells. Vet Immun Immunop *53*, 61-71.

Trichardt, L. (1838). In: Dagboek van Louis Trichardt (1836-1838). Nationale Pers, Cape Town, South Africa.

Uilenberg, G. (1971). Etudes sur la cowdriose a Madagascar. Premiere partie. Rev Elev Méd vét Pays trop *24*, 239-249.

Uilenberg, G. (1982). Experimental transmission of *Cowdria ruminantium* by the Gulf Coast tick *Amblyomma maculatum*: danger of introducing heartwater and benign African theileriasis onto the American mainland. Am J Vet Res *43*, 1279-1282.

Uilenberg, G. (1983). Heartwater (*Cowdria ruminantium* infection): Current status. Adv Vet Sci Comp Med *27*, 427-480.

Uilenberg, G., and Niewold, T. A. (1981). *Amblyomma astrion* Donitz, 1909 (ixodidae), a new experimental vector of heartwater disease. Rev Elev Méd Vét Pays Trop *34*, 267-270.

UNDP/DLS (1993). Enhancing rural capacities through livestock development in The Gambia: project appraisal report.

USAID (1982). Socioeconomic study report on farming systems in The Gambia: Mixed farming project, The Gambia.

Van de Pypekamp, H. E., and Prozesky, L. (1987). Heartwater. An overview of the clinical signs, susceptibility and differential diagnosis of the disease in domestic animals. Onderstepoort J Vet Res *54*, 263-266.

Van Heerden, H., Collins, N. E., Brayton, K. A., Rademeyer, C., and Allsopp, B. A. (2004a). Characterization of a major outer membrane protein multigene family in *Ehrlichia ruminantium*. Gene *330*, 159-168.

Van Heerden, H., Steyn, H. C., Allsopp, M. T. E. P., Zweygarth, E., Josemans, A. I., and Allsopp, B. A. (2004b). Characterisation of the pCS20 region of different *Ehrlichia ruminantium* isolates. Vet Microbiol *101*, 279-291.

Van Kleef, M., Neitz, A. H. W., and De Waal, D. T. (1993). Isolation and characterization of antigenic proteins of *Cowdria ruminantium*. Rev Elev Méd vét Pays trop *46*, 157-164.

Van Vliet, A. H. M., Jongejan, F., and Van der Zeijst, B. A. M. (1992). Phylogenetic position of *Cowdria ruminantium* (Rickettsiales) determined by analysis of amplified 16S ribosomal DNA sequences. Int J Syst Bacteriol *42*, 494-498.

Van Vliet, A. H. M., Jongejan, F., Van Kleef, M., and Van Der Zeijst, B. A. M. (1994). Molecular cloning, sequence analysis, and expression of the gene encoding the immunodominant 32-kilodalton protein of *Cowdria ruminantium*. Infect Immun *62*, 1451-1456.

Van Vliet, A. H. M., Van Der Zeijst, B. A. M., Camus, E., Mahan, S. M., Martinez, D., and Jongejan, F. (1995). Use of a specific immunogenic region on the *Cowdria ruminantium* MAP1 protein in a serological assay. J Clin Microbiol *33*, 2405-2410.

Vercruysse, J., Lafia, S., and Camicas, J. L. (1982). Les tiques (*Amblyommidae*) parasites des bovins en Republique du Benin. Rev Elev Méd vét Pays trop *35*, 361-364.

Viljoen, G. J., Vermeulen, N. M. J., and Neitz, A. W. H. (1987). The theoretical aspects of the enzyme-linked immunosorbent assay technique and its use in the detection of *Cowdria ruminantium* antigen and antibody in reacting animals. Onderstepoort J Vet Res *54*, 305-312.

Viljoen, G. J., Vermeulen, N. M. J., Oberem, P. T., Prozesky, L., Verschoor, J. A., Bezuidenhout, J. D., Putterill, J. F., Visser, L., and Neitz, A. W. H. (1985). Isolation of *Cowdria ruminantium* by cellular affinity chromatography and detection by an enzyme-linked immunosorbent assay. Onderstepoort J Vet Res *52*, 227-232.

Waghela, S. D., Rurangirwa, F. R., Mahan, S. M., Yunker, C. E., Crawford, T. B., Barbet, A. F., Burridge, M. J., and McGuire, T. C. (1991). A cloned DNA probe identifies *Cowdria ruminantium* in *Amblyomma variegatum* ticks. J Clin Microbiol *26*, 2571-2577.

Walker, J. B., and Olwage, A. (1987). The tick vectors of *Cowdria ruminantium* (Ixodoidae, Ixodidae, genus *Amblyomma)* and their distribution. Onderstepoort J Vet Res *54*, 353-379.

Webb, J. (1877). Report of the Disease Commission of the Cape of Good Hope, pp 108-111. Saul Solomon & Co., Cape Town.

Whitelaw, D. D., Scott, J. M., Reid, H. W., Holmes, P. H., Jennings, F. W., and Urquhart, G. M. (1979). Immunosuppression in bovine trypanosomiasis: studies with loupin-ill vaccine. Res Vet Sci *26*, 102-107.

Wiegarg, G., and Remington, S. J. (1986). Citrate synthase: structure, control, and mechanism. Ann Rev Biophys Chem *15*, 97-117.

Wilson, T. R. (1991). Small ruminant production and small ruminants genetic resources in tropical Africa. FAO, Animal Health and Production Paper 70.

Young, A. S., Groocock, C. M., and Kariuki, D. P. (1988). Integrated control of ticks and tick-borne diseases of cattle in Africa. Parasitology *96*, 403-432.

Yunker, C. E. (1996). Heartwater in sheep and goats: a review. Onderstepoort J Vet Res *63*, 159-170.

Yunker, C. E., Byrom, B., and Semu, S. (1988). Cultivation of *Cowdria ruminantium* in bovine vascular endothelial cells. Kenya Vet *12*, 12-16.

Yunker, C. E., Mahan, S. M., Waghela, S. D., McGuire, T. C., Rurangirwa, F. R., Barbet, A. F., and Wassink, L. A. (1993). Detection of *Cowdria ruminantium* by means of a DNA probe, pCS20 in infected bont ticks, *Amblyomma hebraeum*, the major vector of heartwater in southern Africa. Epidemiol Infect *110*, 95-104.

Zweygarth, E. (2006). *In vitro* cultivation of *Ehrlichia ruminantium* and development of a culture-derived attenuated vaccine. PhD Thesis, Utrecht University, Utrecht, The Netherlands.

Zweygarth, E., and Josemans, A. I. (2001). Continuous *in vitro* propagation of *Cowdria ruminantium* (Welgevonden stock) in a canine macrophage-monocyte cell line. Onderstepoort J Vet Res *68*, 155-157.

Zweygarth, E., Josemans, A. I., Van Strijp, F. M., Lopez-Rebollar, L., Van Kleef, M., and Allsopp, B. A. (2005). An attenuated *Ehrlichia ruminantium* (Welgevonden stock) vaccine protects small ruminants against virulent heartwater challenge. Vaccine *23*, 1695-1702.