

Heartwater in sheep and goats: a review

CONRAD E. YUNKER*

Onderstepoort Veterinary Institute, Private Bag X5, Onderstepoort, 0110 South Africa

ABSTRACT

YUNKER, C.E. 1996. Heartwater in sheep and goats: a review. *Onderstepoort Journal of Veterinary Research*, 63:159–170

Heartwater (cowdriosis) is an important, often fatal, tick-borne disease of domestic and wild ruminants in sub-Saharan Africa and some Indian Ocean and Caribbean islands. The causal agent, *Cowdria ruminantium* (Cowdry 1925), is a rickettsia closely related to members of the genus *Ehrlichia*, and is probably a part of a complex of genomic species. Imported breeds of sheep and goats (especially Angoras) are highly susceptible, but indigenous populations of endemic areas may be resistant to infection. Very young stock (less than 9 d old) possess a natural resistance that is unrelated to the immune status of the dams. Symptoms of heartwater vary, but usually begin with fever and may involve neurological signs and respiratory distress. Clinical diagnosis is based on symptoms, history of tick-exposure and post-mortem findings, and is confirmed by demonstration of characteristic rickettsial organisms in vascular endothelial cells. Laboratory diagnosis is retrospective and includes fluorescent antibody and enzyme-linked immunosorbent assays. Serological tests are compromised by non-specific reactions with certain *Ehrlichia* spp. DNA and oligonucleotide probes have been developed, but are thus far unavailable in many countries affected by heartwater. Treatment with tetracyclines is effective if begun in the early stages of infection. Control is based on a knowledge of the disease cycle in nature, and is achieved through judicious tick control, vaccination or both. A virulent, blood-based vaccine is available. Existence of a carrier state in recovered animals, including wild ruminants, complicates control efforts, and eradication is feasible only in circumscribed foci. Problem areas in fundamental and applied research on heartwater, as it affects sheep and goats, are discussed.

Keywords: *Cowdria ruminantium*, cowdriosis, goats, heartwater, rickettsia, sheep

INTRODUCTION

Heartwater (cowdriosis) was known as a disease of sheep and goats for more than six decades before it became recognized as a cattle disease. On 8 March 1838, the South African pioneer, Louis Trichardt, recorded in his journal a fatal disease among his sheep, which he termed "a type of Nintas" (Preller 1938). The disease, characterized by nervous symptoms, was preceded by a massive tick infestation acquired 3

weeks earlier upon descending into the lowveld of eastern Transvaal. Neurological symptoms and death of ruminants following tick bite is typical of heartwater. The eastern Transvaal is characteristically inhabited by the main southern African vector of the disease, *Amblyomma hebraeum*, and the hot, wet season of mid-February is compatible with peak activity of the tick. Thus, Trichardt's diarized notes have been adjudged the first historical record of heartwater (Neitz 1947; Camus & Barré 1982; 1988; Provost & Bezuidenhout 1987).

The disease, observed and anecdotally reported by South African farmers thereafter, was recorded 39 years later by J. Webb, a livestock producer in the Eastern Cape Province. In testimony in 1877 to the

* Present address: Tickconsult, 230 Pioneer Drive, Port Ludlow, WA 98365, USA

Accepted for publication 3 April 1996—Editor

Cape of Good Hope Commission on Diseases of Sheep and Goats (Anonymous 1877; 1898) he noted that the appearance of bont ticks (*A. hebraeum*) on his farm some 8 or 9 years previously, correlated with the onset of fatal disease in stock. On opening the thorax of victims, he found "the heart bag and chest full of water" and surmised that this was due to "inflammation brought on by the tick." He noted that one farmer in the region lost 2 000–3 000 head of sheep and goats, and that another had witnessed the introduction into the Eastern Province of the bont tick on cattle imported from Zululand in 1837.

By the end of the century, confirmation had replaced supposition. Researchers had demonstrated that the disease could be transmitted by subinoculation of blood from sick to healthy animals (Edington 1898; Dixon 1898) and was therefore probably a microbial infection (Hutcheon 1900). Simultaneously, it was indisputably proven that the bont tick was a vector (Lounsbury 1900). These discoveries, as pointed out by Provost and Bezuidenhout (1987), provided the knowledge necessary to reproduce and study the disease in a laboratory situation. However, another quarter of a century was to pass before the causal agent was identified as a rickettsia (Cowdry 1925).

ETIOLOGY

Causal agent

Cowdria ruminantium (Cowdry 1925), "small pleomorphic, coccoid or ellipsoidal, occasionally rod-shaped organisms occurring intracytoplasmically but not intranuclearly, and characteristically localized in clusters inside vacuoles in the cytoplasm of vascular endothelial cells of ruminants" (Ristic & Huxsoll 1984).

Classification and relationships

The heartwater organism, a member of the Class Proteobacteria and Order Rickettsiales, is currently classified in Bergey's Manual as a member of the Family Rickettsiaceae, Tribe Ehrlichieae and Genus *Cowdria* Moshkovski 1947 (Ristic & Huxsoll 1984). *Cowdria* is a monotypic genus erected to contain as type-species *Rickettsia ruminantium* Cowdry 1925. Other genera in the Tribe are *Ehrlichia* Moshkovski 1945 and *Neorickettsia* Philip *et al.* 1953. This classification is based on morphological, biological and distributional characters. Recently, analyses of 16S ribosomal RNA sequences from two isolates of *C. ruminantium* have been reported (Dame, Mahan & Yowell 1992; Van Vliet, Jongejan & Van der Zeijst 1992). Comparison of these sequences with those from other Proteobacteria has demonstrated a closer relationship than previously thought, of *C. ruminantium* with certain *Ehrlichia* spp. (Van Vliet *et al.* 1992) and *Anaplasma marginale* (Dame *et al.* 1992), and a

greater phylogenetic distance of *Cowdria* from *Chlamydia* (Order Chlamydiales) than suggested by numerical taxonomic analysis (Scott 1987). Although the genotypic findings do not mandate a change in the formal taxonomic position of the heartwater organism (Ristic & Huxsoll 1984), they do imply that the current organization of the Order Rickettsiales be reviewed as small subunit rRNA (srRNA) sequences for additional Proteobacteria become known (Van Vliet *et al.* 1992).

Allsopp & Visser (1994) have amplified and sequenced a part of the srRNA gene of several stocks of heartwater obtained from infected animals, and found a totally unexpected degree of heterogeneity. Only one isolate yielded a single sequence related to *Cowdria ruminantium*, but others yielded many, suggesting the existence of multiple genomic species in the genus *Cowdria*. The possibility that heartwater might be a disease of multiple etiologies had already been suggested, based on phenotypic characteristics (Yunker 1991).

DISTRIBUTION AND VECTORS

Distribution

Heartwater is a disease of global importance, being established in both hemispheres and having a potential for introduction into many countries to which vectors may be transported or where they already occur. Distribution of the disease has been reviewed by Camus & Barré (1982; 1988). It is widespread in Africa (and some adjacent islands) south of the Sahel Transitional Zone (White 1983), and probably occurs in all African countries in which the vectors, *Amblyomma* spp. excluded, are established. A single sub-Saharan country, Lesotho, is presumed to be heartwater-free by virtue of its high altitude and cold climate, which is inhospitable to *Amblyomma* ticks (Yunker & Allsopp 1994). Also excluded from the range of the disease, are large desert and semi-desert areas of Somalia, southern Angola, Botswana, Namibia, and western and south-central South Africa. Elsewhere, heartwater is known from Indian Ocean islands of the Comores (Bezuidenhout, Olivier, Kruger, Lombard & Du Plessis 1989), Madagascar (Poisson & Geoffroy 1925; Buck 1965) and the Mascarenes, Reunion and Mauritius (Perreau, Morel, Barré & Durand 1980), where a major vector, *A. variegatum*, is established. The latter tick, transported through the activities of man to the Antillean islands of the New World in the last century, has spread to a number of eastern Caribbean islands and heartwater is endemic in at least three of them: Guadeloupe, Antigua and Marie Galante (reviewed by Uilenberg 1990a). The spread of this vector correlates with recent expansion of the range of the cattle egret, *Bubulcus ibis*, in that area, and it appears to be only a matter of time before vector and disease

are transported to the continental land masses where the potential for damage to the livestock industry is inestimable. Heartwater has been reported or suspected in a number of countries other than the above, but these represent only transient introductions in imported livestock, or are unconfirmed (Camus & Barré 1982; 1988).

Vectors

Although *Amblyomma* spp. are known from many parts of the world, vectors of heartwater in nature are all of African origin (Walker & Olwage 1987). Members of this tropical and subtropical genus are all three-host ticks that have a wide host range, especially in the immature stages. Host preferences of the various species influence their importance as vectors (Petney, Horak & Rechav 1987). Sheep and goats are important hosts for immatures of the two principal vectors, *A. hebraeum* (southern Africa) and *A. variegatum* (the rest of Africa and elsewhere), which commonly utilize cattle as adults. Other species (i.e. *A. astrion*, *A. cohaerens*, *A. gemma*, *A. lepidum* and *A. pomposum*) are secondary in importance because of limited distribution on or infrequent parasitism of domestic stock. A third category, accidental vectors, consists of species that do not normally feed on domestic stock: *A. marmoreum*, *A. sparsum* and *A. tholloni*. In the New World, in addition to *A. variegatum*, which is thus far limited to the Lesser Antilles, three potential (experimentally proven) vectors are known: *A. cajennense*, *A. maculatum* and *A. dissimile* (Uilenberg 1982; 1983; Jongejan 1992a).

ECONOMIC IMPORTANCE

For most of this century, heartwater has been considered one of the most important livestock diseases in Africa (Maré 1984). In regions of Africa free of East Coast Fever (ECF) and tsetse-borne trypanosomiasis, it is considered the most important vector-borne disease of cattle (Uilenberg 1983). In western and southern Africa, it is at least one of the most important tick-borne diseases, not only because of its capacity for causing severe losses in cattle, but because here, in contrast to other major tick-borne diseases of cattle, such as anaplasmosis, babesiosis and theileriosis, heartwater is of considerable economic importance in sheep and goats (Norval, Andrew, Yunker & BurrIDGE 1992). However, few factual estimates of the economic impact of heartwater with which to support such beliefs are available. Bigalke (1983) reported that losses from heartwater in South Africa during the period 1981-82, represented 30,3% of 1,53 million head lost to the three major tick-borne diseases, heartwater, anaplasmosis and redwater. The cost incurred from losses due to heartwater was estimated to lie between 21,2 and 54,5 million Rand. Du Plessis, De Waal & Stoltz (1994) surveyed the

incidence and importance of tick-borne diseases in heartwater-endemic areas of South Africa and presented data that show losses in livestock from heartwater to greatly outnumber those from redwater and gallsickness combined. Furthermore, mortality due to heartwater was three times higher in sheep and goats than in cattle.

HOSTS AND SUSCEPTIBILITY

Domestic livestock

As late as 1902, heartwater was thought to be a specific disease of sheep and goats, which were "the only animals known to naturally contract heartwater" (Lounsbury 1902).

However, cattle along with sheep and goats, are classical victims. [In fact Louis Trichardt's oxen became sick within two days of his sheep dying. Although he associated the bovine affliction with (tsetse) flies and subsequent authors have attributed it to nagana (Fuller 1924), heartwater may also have been involved.] Domestic (Asian) buffalo (*Bubalis bubalis*) are also receptive and can die from heartwater (Mammerickx 1961). There is no conclusive evidence for susceptibility of the camel to heartwater (Uilenberg 1983), and other domestic animals are apparently insusceptible.

Wild animals

A large number and variety of African and non-African ruminants and other animals are susceptible to infection with heartwater (Uilenberg 1983; Oberem & Bezuidenhout 1987), giving rise to speculation that some, in heartwater-endemic areas, may serve as reservoirs of the disease. The controversy has been resolved with the finding that African buffalo (*Synceiros caffer*), which serve as excellent natural hosts for the vectors are, after recovery from heartwater, chronically infected and intermittently infective for ticks for many months, and perhaps permanently (Andrew & Norval 1989). The range of susceptible vertebrates and potential carriers is not limited to ruminants, but includes birds and reptiles as well. Both guinea fowl and leopard tortoises can be infected; they respond subclinically and transfer the infection to *Amblyomma* ticks, which feed on these hosts in nature (Bezuidenhout & Olivier 1986). The participation of wild animals in the cycle of heartwater, complicates efforts at control (Uilenberg 1983) but may also serve to maintain a state of endemic stability.

Laboratory animals

The discovery of a unique stock of *Cowdria* that was infective for Swiss mice (Du Plessis & Kümm 1971) provided, for the first time, a laboratory model for

research in diagnosis, immunology, pathogenesis, epidemiology and control of the disease. Since then, a number of *Cowdria* isolates have been found to infect laboratory mice, whereas others are non-infective (reviewed by MacKenzie & McHardy 1987).

Susceptibility and resistance

Animals exposed to heartwater respond in a variety of ways, depending on a number of factors, including species, breed, age, immune status and stock of *Cowdria*. Exotic breeds of sheep and goats are of uniformly high susceptibility and mortalities of 50% or greater due to heartwater have been seen in sheep and cattle imported into sub-Saharan Africa (Uilenberg 1983; 1989). Merino sheep are highly susceptible (Lounsbury and Robertson 1904; Alexander 1931). Goats, particularly Angoras, are the most receptive to heartwater of all domestic ruminants (Spreull 1922), with mortalities surpassing 90% in imported stock (Uilenberg 1989). In contrast, annual mortality in indigenous goats of an endemic area (Guadeloupe) has been estimated at around 10% (Camus 1987). Wild ruminants may either be refractory or receptive to infection, but of 11 species of non-African ruminants tested, all responded to infection with clinical signs and/or death (Oberem and Bezuidenhout 1987).

The innate resistance of indigenous breeds in endemic areas has not been fully examined and, in many instances, it is not possible to judge whether resistance is a characteristic of the breed, or if the resistant state has been acquired through natural selection that favours survival of the least susceptible animals upon prolonged exposure (Uilenberg 1983). It was recognized very early that sheep in South Africa presumed to be Persian were markedly resistant to infection (reviewed by Lounsbury and Robertson 1904). [The identity of the breed is questionable and it may have originated within the endemic area of heartwater (Uilenberg 1981). Lounsbury and Robertson (1904) state that the breed was first introduced into South Africa in 1872 from a shipment originating in the "Mediterranean", and a subsequent introduction (1884) originated in "Arabia." However, the first "Persians" imported into South Africa were either Somali desert sheep or Hedjaz fat-tailed sheep from Saudi Arabia (Anon 1965). Regardless, the term: Persian, as applied to South African sheep, is a misnomer, the breed being introduced from various Red Sea ports.] Numerous instances of these "Persians" remaining healthy while grazing in tick-infested veld, along with highly susceptible Merino sheep, and Boer and Angora goats that were seriously affected, are recorded (Lounsbury and Robertson 1904). Likewise, natural resistance to heartwater is a pronounced feature of the indigenous Afrikaner sheep (Alexander 1931).

In South Africa, Donkin, Stewart, Macgregor, Els & Boyazoglu (1992), compared resistance to heartwa-

ter of Saanen goats with that of indigenous and Saanen:indigenous (50:50) crosses. After infection, all of eight Saanen goats died, but seven of eight indigenous and six of eight crossbred goats survived. In addition, the severity of clinical signs recorded was greater in the Saanens than in the indigenous goats and crosses. These results indicate that indigenous goats possess a resistance to heartwater that can be transmitted to crossbred offspring. It had previously been shown that Creole goats on Guadeloupe are resistant to severe (but not mild) heartwater manifestations (Matheron, Barré, Camus & Gogue 1987). The resistance varied from flock to flock, depending on its history of exposure to heartwater. The rate declined progressively when animals had been reared in disease-free areas. Male goats were significantly more resistant than females, and paternity influenced the resistant status of the kids. This resistance, apparently under control of a sex-linked recessive gene, suggests that selection for the trait is feasible. Efforts are in progress to determine the genetic basis for this resistance (Ruff, Maillard, Camus, Depres & Matheron 1993; Pepin, Camus, Matheron & Bensaid 1993).

Newborn and very young stock possess an innate resistance to heartwater that diminishes with age (Neitz & Alexander 1941). This particular type of resistance, which is independent of the susceptibility or immune status of the dam, is short-lived in lambs (7–9 d), longer in calves (3–4 weeks) and, although present in kids, its duration is not established (Thomas & Mansvelt 1957; Uilenberg 1983).

SYMPTOMS AND COURSE OF INFECTION

The incubation period in sheep and goats is variable and may range from five to 35 d (Alexander 1931) but in most cases symptoms first appear between seven and 14 d post-inoculation, commencing on average on the ninth day. In naturally acquired infections, symptoms are seen after between nine and 28 d and the average incubation period is 12 d (Neitz 1968). Fever and inappetence, or fever alone, usually occurs first, followed by a variety of symptoms, depending on the course of the disease. Mortality rates are high in sheep and goats, and both average incubation period and virulence vary with the strain of *Cowdria* (Uilenberg 1983).

Subclinical infections

Mild or latent infections, evinced by a marked pyrexia and followed within 2–3 d by complete recovery, are known (Alexander 1931). The incidence of this form is unknown owing to the inapparent symptoms (Van de Pypekamp & Prozesky 1987), but should be substantial. This course is seen in some indigenous populations in endemic areas, very young animals, immune or partially immune animals and treated stock

(Yunker 1991). Recovered sheep, goats and cattle, as well as African buffalo, become long-term carriers (Andrew & Norval 1989; Camus 1992).

Per-acute infections

This form of infection is characterized by sudden death some 36 h after clinical symptoms first appear (Alexander 1931). Symptoms are of a fulminating temperature in an animal with otherwise normal appearance and behaviour, followed by paroxysmal convulsions, respiratory distress and sudden collapse. Both goats and sheep are vulnerable, but per-acute cases are more common in the former (Uilenberg 1983).

Acute infections

This is the classical, most common form of infection in susceptible animals (Alexander 1931). Pyrexia of several days duration, subsiding when death approaches, is followed by listlessness, rapid pulse and tachypnoea, and a disturbed expression in the eyes (Neitz 1968). Nervous symptoms ensue, such as a twitching of facial muscles and high stepping gait (Alexander, Neitz & Adelaar 1946). Sheep and goats show a progressive incoordination and stand in an attitude with head down, ears drooping, and thorax heaving. Hydrothorax (but seldom hydropericardium) may be established by percussion. Following this, the animal often collapses and lies on its side with its head thrown back, while showing strabismus, galloping movements of the legs, masticatory and licking movements of the mouth, and frothing from the nostrils. Feeding frequently continues until shortly before collapse. The temperature shows an abrupt fall to subnormal, prior to death. Clinical signs may last 3–5 d and, in animals that go down, recovery is rare. Recovered sheep may shed part or all of the fleece.

Subacute infections

Symptoms here resemble those seen in acute cases, but are less pronounced (Alexander 1931; Neitz 1968). Fever may persist for 10–15 d, and pregnant animals are prone to abort. Animals may collapse and die, with death often due to complications, such as pneumonia or sequelae resulting from atony of the fore stomachs, otherwise a gradual subsidence of symptoms followed by recovery may occur.

PATHOLOGY

The post-mortem lesions and histopathology of numerous cases of heartwater in sheep, goats and cattle were reported in detail by Steck (1928) and Alexander (1931). These findings were reviewed and augmented by Uilenberg (1983) and Prozesky (1987). Most lesions are restricted to acute and su-

bacute forms of infection, and may vary according to the stock of *Cowdria* involved.

Post-mortem pathology

Lesions among domestic ruminants are similar, with some exceptions. Effusion of body cavities, especially thoracic cavities, is commonly seen in fatal cases, amounting to 0.5 l or more in sheep, but barely more than 20 ml in goats. Hydropericardium (from which the disease gets its name) is a regular finding, and is more pronounced in sheep and goats than in cattle. The lungs, mediastinum and associated lymph nodes are oedematous and a serous, frothy fluid oozes from cut surfaces of the lungs. In peracute cases, marked oedema of the lungs, and froth in the trachea and bronchi are striking enough to explain death by asphyxia (Uilenberg 1981).

Splenomegaly occurs in over 90% sheep and goats (Steck 1928), but enlargement may not be marked. Other workers have not frequently observed splenomegaly in small ruminants (Uilenberg 1971b; Prozesky 1987). The lymph nodes are swollen and the kidneys somewhat pale. The liver is congested, often showing fatty degeneration (Neitz 1968), but hepatic lesions are not striking (Prozesky 1987). Enteritis is seen less often in small stock than in cattle. Except for congestion of meningeal vessels or occasional meningeal oedema, macroscopic brain lesions are seldom seen.

Histopathology

Lesions of most organs are not remarkable (Steck 1928) and particular attention has been paid to the brain. Here, swollen axis cylinders, multifocal microcavitations and haemorrhages affecting mainly the midbrain, cerebral white matter and peduncles, are seen (Pienaar, Basson & Van der Merwe 1966; Prozesky 1987). Often, leucostasis of small vessels and, sometimes, mononuclear perivascular infiltration is noted.

Swollen, often necrotic, astrocytes frequently seen in cattle, are seldom observed in sheep and goats. Leucostasis is also pronounced in kidneys, liver, lungs and adrenals. Nephrosis is seen in over 50% of ruminants examined (Steck 1928), but multifocal, lymphocytic, interstitial nephritis seen by Steck was not confirmed by subsequent investigators (Prozesky 1987). In lungs, an alveolar and interstitial oedema frequently occurs, but this may be obliterated during tissue processing (Prozesky 1987).

CLINICAL DIAGNOSIS

Presumptive diagnosis is based on clinical signs, history of tick exposure, and post-mortem findings. Heartwater must be differentiated from certain plant

and mineral poisonings, as well as a number of infectious viral, bacterial and parasitic diseases (Uilenberg 1983). It is confirmed by the finding of characteristic colonies of the causal agent in capillary endothelial cells, particularly of brain smears (Purchase 1945), or by subinoculating blood of sick animals into susceptible ruminants. Brain smears are usually done at autopsy, but can be made from the living animal by needle biopsy (Synge 1978); however, this is impractical on a routine basis for a number of reasons (Uilenberg 1994). Confirmation by subinoculation is slow and expensive, and ultimately depends on demonstration of rickettsial organisms in endothelial cells.

LABORATORY DIAGNOSIS

Diagnostics for cowdriosis have been the subject of a number of recent reviews (Camus 1994; Du Plessis, Bezuidenhout, Brett, Camus, Jongejan, Mahan & Martinez 1993; Jongejan 1992b; Uilenberg 1994; Yunker 1991; Yunker and Allsopp 1994). Most diagnostics are retrospective and none have been developed in kit form for use in the field or provincial laboratory situation. The most promising of these (DNA and oligonucleotide probes, PCR amplification techniques) are sufficiently high tech as to preclude their use in the laboratories of many countries affected by heartwater.

Soerological tests

These include indirect fluorescent antibody (IFA), enzyme-linked immunosorbent assay (ELISA) and Western Blots. However, all serological tests developed to date show non-specific reactions with one or more *Ehrlichia* spp., hence are of limited value.

IFA tests may be performed with antigens produced either *in vivo* in mouse macrophages (Du Plessis & Malan 1987a) or in caprine neutrophils (Logan, Holland, Mebus & Ristic 1986; Logan, Whyard, Quintero & Mebus 1987), or *in vitro* (see below). In South Africa, the mouse macrophage IFA test is used both as a research tool and a confirmatory diagnostic for sera found negative in the ELISA test for heartwater. Its sensitivity for heartwater is higher than that of other IFA tests, and it has a greater capacity to reduce non-specific background fluorescence (Du Plessis *et al.* 1993). Thus it is capable of detecting lower levels of antibody, hence useful for epidemiological studies. Because of the need to maintain mouse colonies, it is usually suitable only for centralized laboratories or research institutions. Consequently, it has not been widely used. The caprine neutrophil IFA test, which is not suitable for all stocks of *Cowdria* (Jongejan 1992a/b), is expensive as well as difficult to reproduce; hence it is not employed as a routine diagnostic.

The advent of an *in vitro* system for the growth of *Cowdria ruminantium* in endothelial cells (Bezuidenhout *et al.* 1985) permitted the production of large quantities of relatively pure antigen which could be used in the IFA test (Yunker, Byrom & Semu 1988; Martinez, Swinkels, Camus & Jongejan 1990; Semu, Mahan, Yunker & Burr ridge 1992). This test is appropriate for most diagnostic laboratories, as long as one keeps in mind its inability to discriminate between *Cowdria* and *Ehrlichia* antibodies. It has been used in an epidemiological study of heartwater reactors among goats and cattle of Mozambique (Asselbergs, Jongejan, Langa, Neves & Afonso 1993), and also to confirm the immune status of vaccinated calves in Malawi (Lawrence, Whiteland, Malika, Kafuwa & Jongejan 1993).

With the availability of cultured organisms, a number of ELISA tests have been developed that employ as antigens purified, whole or sonicated elementary bodies of *Cowdria* or proteins solubilized from the elementary bodies (Jongejan, Thielemans, Van Kooten, De Groot & Van der Zeijst 1991b; Jongejan 1992b; Soldan, Norman, Masaka, Paxton, Edelsten & Sumption 1993). A competitive ELISA (cELISA), employing monoclonal antibodies directed against the immunodominant 32-kiloDalton protein of *Cowdria*, which is conserved in all stocks tested to date, has been used to investigate the distribution of heartwater on 19 islands of the Caribbean (Muller Kobold, Martinez, Camus & Jongejan 1992). The gene encoding this protein has been cloned and expressed in *Escherichia coli* for use as antigen in cELISA tests (Jongejan, Van Vliet & Van der Zeist 1994). Meanwhile, epitope mapping of this recombinant antigen is being carried out in attempts to exclude epitopes that give false positive reactions.

Western-blot assays have been used to identify antibodies to *Cowdria* in field-collected specimens (Muller Kobold, Martinez, Camus & Jongejan 1992; Barbet, Tebele, Semu, Peter, Wassink & Mahan 1993; Mahan, Tebele, Mukwedeya, Semu, Nyathi, Wassink, Kelley, Peter & Barbet 1993b), but are used mainly in experimental work and for validating results of other diagnostic studies (reviewed by Yunker & Allsopp 1994).

Repetitive DNA probe

A DNA probe, pCS20, has been made from total DNA of *Cowdria*-infected bovine endothelial-cell cultures and used to identify *Cowdria* DNA in ticks (Waghela, Rurangirwa, Mahan, Yunker, Crawford, Barbet, Burr ridge 1991; Yunker, Mahan, Waghela, McGuire, Rurangirwa, Barbet & Wassink 1993) and sheep plasma (Mahan, Waghela, McGuire, Rurangirwa, Wassink & Barbet 1992a). The probe hybridizes with all *Cowdria* stocks thus far tested, including one from the Caribbean, and it was possible to detect the

organism in infected sheep before the onset of fever, which is the first clinical sign. The pCS20 probe was highly specific, failing to hybridize with DNA from 22 other pro- or eukaryotic species: cattle, ticks (two), protozoa (three), rickettsiae (four), and bacteria (12), including *Anaplasma marginale*, *Rickettsia* spp., *Chlamydia* spp., and *Ehrlichia risticii*. However, it remains to be tested for cross-reactivity with certain *Ehrlichia* spp. that give false positive reactions in serological tests. It also requires conversion to a non-radioactive method of detection of hybridization for use in field situations or provincial laboratories.

Oligonucleotide probes

Recently, srRNA partial gene sequence analyses of several African isolates obtained from heartwater cases has revealed considerable genotypic heterogeneity within the species nominally known as *C. ruminantium* (Allsopp & Visser 1994). While some isolates yielded only a single sequence, others comprised several different ones. Work is underway at the Onderstepoort Veterinary Institute to design oligonucleotide probes capable of identifying individual genotypes among this multiplicity of new organisms. Further studies into this aspect of heartwater etiology will undoubtedly affect nomenclature and concepts of the disease, as well as improve diagnostics and vaccine strategies.

PCR amplification

Although sensitive enough to detect 1 ng of *Cowdria* DNA, sensitivity of the PCS20 DNA probe can be improved by polymerase-chain-reaction (PCR) amplification of *Cowdria*-specific sequences, which may be necessary to detect animals having a low rickettsemia, such as heartwater carriers (Mahan, Waghele, Rurangirwa, Yunker, Crawford, Barbet, Burrige & McGuire 1992b).

TREATMENT

Various formulations of the tetracyclines are almost invariably used for treatment of heartwater (Purnell 1984; Van Amstel & Oberem 1987). These are administered at the rate of 8–10 mg/kg or, in the case of doxycycline, at 2 mg/kg (Immelman & Dreyer 1982). Two injections on consecutive days, or on the first and third day after onset of fever, are advisable (Uilenberg 1983). Intravenous, intramuscular and oral routes of administration are effective. Treatment of small stock showing clinical signs, has resulted in a 48 % recovery rate (Du Plessis *et al.* 1994). Uleron, the first agent found to be effective in treating heartwater in sheep (Neitz 1940), is one of many sulphonamides used with good results. However, repeated doses may be necessary, and it is believed that tetracyclines are more effective (Van Amstel & Oberem 1987).

EPIDEMIOLOGY AND CONTROL

Control is based on a knowledge of the disease cycle. In the field, heartwater is seen some weeks after susceptible stock are subjected to tick-challenge. This occurs when they are introduced into an endemic area for the first time (particularly just prior to or during the rainy season), when vectors encroach into new areas, or after an intensive tick-control programme has been interrupted and ticks are reintroduced through movements of wild game or undipped stock. Young animals that have not been exposed to ticks until after their brief period of innate immunity has disappeared, are particularly vulnerable. On the other hand, their exposure to ticks during the first week of life may insure immunity.

Preventative measures

Control is usually achieved through tick control or vaccination. The decision as to which approach to use, requires that the question of endemic stability/instability first be resolved. Eradication is, at present, feasible only in circumscribed foci such as certain islands, endemically unstable or climatically marginal areas, or in situations where heartwater carriers and hosts for vectors are excluded. The use of indigenous, resistant breeds is recommended for areas lacking adequate tick control and veterinary care (Uilenberg 1990b).

In endemically stable areas where tick control is not practised, a high level of immunity in stock, particularly cattle, is typically seen. Here, if acaricidal programmes are instituted and rigidly enforced (intensive control), the level of herd immunity that results from exposure to ticks, will decline (Howell, De Vos, Bezuidenhout, Potgieter & Barrowman 1981). Stock will then be highly susceptible and at risk, should control break down for any reason. Hence, intensive control may actually increase losses due to heartwater. Selective control (i.e., acaricidal application only when the level of infestation causes tick-worry or hide damage) tends to reinforce endemic stability, and herds may develop a high degree of immunity. High infection rates of vectors, such as reported for *A. hebraeum* in Zimbabwe (Norval, Andrew & Yunker 1990), would tend to insure exposure, hence induce immunity, particularly of young stock temporarily protected by age-related resistance. However, there is some doubt as to whether the concept of endemic stability is as valid for management of heartwater in small ruminants as it is in cattle (H.R. Andrew, personal communication 1992). This may reflect the host preferences of adult *Amblyomma* ticks, which are less likely to be found on small stock than on cattle.

In unstable or transitional areas, where tick populations are subject to climatological extremes, where intensive control has been practised, or where wild hosts are excluded, tick numbers may be insufficient

to maintain immunity in livestock. Also, areas where vector populations [e.g., *A. variegatum* (Uilenberg 1971a; Camus & Barré 1987)] are permanently established, but not highly infected may be endemically unstable. In such cases, intensive tick control (Howell, *et al.* 1981; Bezuidenhout & Bigalke 1987) or immunization (Norval *et al.* 1992) are recommended.

Immunization is accomplished by infecting animals with a virulent preparation of *C. ruminantium* in sheep blood (Neitz & Alexander 1941; 1945) and treating the infection with anti-rickettsial drugs such as tetracyclines (reviewed by Uilenberg 1983; Oberem & Bezuidenhout 1987). Choice of the vaccine stock is an important consideration because of significant antigenic and immunogenic differences among stocks (Logan, Birnie & Mebus 1987b; Du Plessis, Van Gas, Olivier & Bezuidenhout 1989; Jongejan, Thielemans, Briere & Uilenberg 1991a). In addition, susceptible breeds of goats, in particular, may suffer significant mortalities after immunization and treatment (Du Plessis, Jansen & Prozesky 1983; Gruss 1983; Uilenberg 1989). From an environmental standpoint, the wisdom of introducing a standard vaccine strain into an area where it is not known to exist, is questionable, and it might be safer to prepare vaccines by using locally prevalent isolates (J.A. Lawrence, personal communication 1990).

The vaccine is administered intravenously to sheep and goats in 5-ml amounts and the animals are monitored for onset of fever, after which they are treated. With Ball-3 vaccine stock, sheep may be safely treated on the 2nd or 3rd day after onset of fever, thus insuring an adequate immune response (J.L. du Plessis, personal communication 1995). Some immediate losses are to be expected owing to peracute reactions and, later, as a result of vaccine failures. Young lambs and kids (under 3 weeks old) are usually not monitored after immunization, but losses, especially in kids, can be expected (Uilenberg 1983). Where daily temperatures cannot feasibly be monitored, the antibiotic may be administered by the block method (reviewed by du Plessis & Malan 1987b). Here, small stock are treated, without reference to febrile reactions, on the 11th (sheep) or 12th (goats) day after vaccination. However, recent experience has shown that Angora goats should not be treated by the block method (J.L. du Plessis, personal communication 1995) but, like other valuable animals, should be monitored for rise in temperature, then treated.

Long-term immunity may be conferred to young stock by exposing them to infected ticks, rather than through vaccination. This occurs when the animals are first introduced into endemic stable areas, especially during times of tick activity. Here, a short series of injections of tetracyclines during the first month of exposure, which gives protection against frank disease while allowing immunity to develop, has

shown promise in cattle (Purnell 1984), and may also be applicable to sheep and goats (except, possibly, Angoras). Adequate exposure to rickettsia, through heavy tick challenge, is probably necessary to insure thorough herd immunity (Du Plessis & Malan 1987b; Purnell 1987).

DISCUSSION

The need for studies in fundamental areas of heartwater research, including immunology, epidemiology, pathogenesis and natural resistance, was outlined by Uilenberg (1990b). To this should be added etiology. Technological advances have made it possible to identify a number of genetic sequences of Proteobacteria in blood from heartwater cases, which sequences were not present in healthy animals (Allsopp & Visser 1994). Although many of these may reflect adventitious, opportunistic infections, five were *Cowdria*-related, and it is reasonable to believe that others remain to be discovered. Therefore, heartwater may be caused by a complex of species, which could account for differences and variations observed in many studies (e.g., antigenic strain differences, limited cross-protection, variable pathogenicity and course of infection, heritability of resistance, etc.). Basic investigations of the genotypic complexity of the agent(s) of heartwater and their phenotypic characteristics are essential to the development of improved diagnostics and vaccines, and should be a priority.

There is a lack of basic information from the field, especially with regard to small stock. The epidemiology of heartwater in sheep and goats is poorly understood, as most information relates to cattle. The latter are hosts to adult ticks, whereas the former are predominantly parasitized by immatures, which would result in a different transmission pattern and affect endemic stability. Infestation rates of small stock with ticks, as well as infection rates of the ticks in various areas, should be determined at regular intervals. The economics of heartwater in small ruminants have also been vastly under-reported, particularly in remote areas and in countries where animals that die, are customarily consumed rather than made available for autopsy. Adequate information on the economic impact of diseases is vital to continued research support.

Other critical problems exist in more applied areas, including diagnosis and control. The non-specificity of serological tests has been addressed by a number of workers in a recent consultation (see section on Laboratory Diagnosis). For example, false positive immunoblot reactions for cowdriosis have been recorded in ruminants in heartwater-free areas of Zimbabwe (Mahan *et al.* 1993b). Similarly, Boer goats in a number of areas of South Africa have tested mostly positive in the ELISA test employing *Cowdria* anti-

gen (S. Vogel, personal communication 1995). The areas in which the goats had been born and reared, were free of both heartwater and amblyomma ticks. Apparently antibodies to some unknown agent, probably a species of *Ehrlichia* transmitted by another kind of tick, had falsely indicated the presence of heartwater. This is especially relevant to the certification for export of domestic and wild ruminants and embryos.

Limitations of the existing heartwater-infected blood vaccine are numerous. It is expensive to produce and store, cumbersome to transport and administer, highly strain-specific and potentially dangerous. In endemic areas of South Africa, only an estimated 15% of farmers raising sheep and goats vaccinate their animals, and those that do suffer higher losses than those who do not (Du Plessis *et al.* 1994). An improved vaccine is clearly needed. Yet, nearly a decade after first cultivation of the heartwater organism (Bezuidenhout *et al.* 1985), which resulted in the production of DNA expression libraries (Ambrosio, Du Plessis & Bezuidenhout 1987; Barbet, Mahan, Allred, McGuire, Palmer & Yunker 1992), no new-generation, genetically engineered vaccine has appeared. Obstacles to heartwater-vaccine development include failure to identify protective epitopes and resolution of the problem of limited cross-protection among stocks.

Finally, innovative approaches to control or avoidance of ticks and heartwater, such as pasture management (including wildlife exclusion), biological control and development of host resistance through cross-breeding or transgenic technology (Stiller 1990) should receive more attention than has been heretofore accorded.

ACKNOWLEDGEMENTS

I thank Drs J.L. du Plessis, L. Prozesky [Onderstepoort Veterinary Institute (OVI)] and Dr H.R. Andrew, Gutu, Zimbabwe, for reading the manuscript and providing helpful comments. Dr S. Vogel, OVI, kindly made available a number of obscure references, as well as unpublished information. Mr E.F. Donkin and Dr N.R. Bryson, Medical University of Southern Africa (MEDUNSA), generously provided documentation concerning South African goats.

REFERENCES

- ALEXANDER, R.A. 1931. Heartwater: The present state of our knowledge. *17th Report of the Director of Veterinary Services and Animal Industry*, Union of South Africa: 89–150.
- ALEXANDER, R.A., NEITZ, W.O. & ADELAAR, T.F. 1946. Heartwater. *Farming in South Africa*, August: 548–552.
- ALLSOPP, B.A. & VISSER, E.S. 1994. Genetic heterogeneity of the causative agent of heartwater. *Proceedings of the 9th International Veterinary Hemoparasite Diseases Conference, Merida, Mexico, 6–9 October 1993* (in press).

- AMBROSIO, R., DU PLESSIS, J.L. & BEZUIDENHOUT, J.D. 1987. The construction of genomic libraries of *Cowdria ruminantium* in an expression vector, Lambda gt11. *Onderstepoort Journal of Veterinary Research*, 54:255–256.
- ANDREW, H.R. & NORVAL, R.A.I. 1989. The carrier status of sheep, cattle and African buffalo recovered from heartwater. *Veterinary Parasitology*, 34:261–266.
- ANONYMOUS. 1877. (Testimony of J. Webb) *Report of the Colonial Veterinary Surgeon, Sheep & Cattle Diseases. Colony Cape of Good Hope*. Appendix: 108–111.
- ANONYMOUS. 1898. Spread of ticks and prevalence of heartwater. *Agricultural Journal of the Cape of Good Hope*, 13:594–595.
- ANONYMOUS. 1965. The blackhead Persian in South Africa. *Farming in South Africa*. January: 34–39.
- ASSELBERGS, M., JONGEJAN, F., LANGA, A., NEVES, L. & AFONSO, S. 1993. Antibodies to *Cowdria ruminantium* in Mozambican goats and cattle detected by immunofluorescence using endothelial cell culture antigen. *Tropical Animal Health and Production*, 25:144–150.
- BARBET, A., MAHAN, S., ALLRED, D., MCGUIRE, T.C., PALMER, G.H. & YUNKER, C.E. 1992. *Recent Developments in the Control of Anaplasmosis, Babesiosis and Cowdriosis*. Publication: ILRAD, Nairobi: 87–91.
- BARBET, A., TEBELE, N., SEMU, S., PETER, T., WASSINK, L. & MAHAN, S. 1993. Serological diagnosis of heartwater in Zimbabwe. Problems and perspectives. *Revue d'Élevage et de Médecine Vétérinaire des Pays Tropicaux*, 46:121 (Abstract only).
- BEZUIDENHOUT, J.D., PATTERSON, C.L. & BARNARD, B.H.J. 1985. *In vitro* cultivation of *Cowdria ruminantium*. *Onderstepoort Journal of Veterinary Research*, 52:113–120.
- BEZUIDENHOUT, J.D. & BIGALKE, R.D. 1987. The control of heartwater by means of tick control. *Onderstepoort Journal of Veterinary Research*, 54:525–528.
- BIGALKE, R. 1985. Onderstepoort today, yesterday and tomorrow. Commemorative lecture. *Onderstepoort Journal of Veterinary Research*, 52:121–132.
- CAMUS, E. 1992. Le portage asymptomatique de bovins et chèvres Créole guéris de la cowdriose en Guadeloupe. *Revue d'Élevage et de Médecine Vétérinaire des Pays Tropicaux*, 45: 133–135.
- CAMUS, E. 1994. Application and uses of specific methods for diagnosis of cowdriosis in developing countries. Present and future trends. *Proceedings of a FAO Expert Consultancy, Use of Applicable Biotechnological Methods for Diagnosing Haemoparasites, Merida, Mexico, 4–6 October 1993* (in press).
- CAMUS, E. & BARRÉ, N. 1982. La cowdriose (heartwater). *Revue Général des connaissances. Institut d'Élevage et de Médecine Vétérinaire des Pays Tropicaux, Maisons-Alfort, France*.
- CAMUS, E. & BARRÉ, N. 1987. Epidemiology of heartwater in Guadeloupe and in the Caribbean. *Onderstepoort Journal of Veterinary Research*, 54:419–426.
- CAMUS, E. & BARRÉ, N. 1988. Heartwater. A review. (A translation of Camus & Barré 1982.) *Publications of the Office International des Epizooties Bulletin*, Paris.
- COWDRY, E.V. 1925. Studies of the etiology of heartwater. I. Observation of a rickettsia, *Rickettsia ruminantium* (n. sp.), in the tissues of infected animals. *Journal of Experimental Medicine*, 42:231–252.
- DAME, J.B., MAHAN, S.M. & YOWELL, C.A. 1992. Phylogenetic relationship of *Cowdria ruminantium*, agent of heartwater, to *Anaplasma marginale* and other members of the order Rickettsiales determined on the basis of 16S rRNA sequence. *International Journal of Systematic Bacteriology*, 42:270–274.
- DIXON, R.W. 1898. Heartwater experiments. *Agricultural Journal of the Cape of Good Hope*, 12:754–762.

- DONKIN, E.F., STEWART, C.G., MACGREGOR, R.G., ELS, H.C. & BOYAZOGLU, P.A. 1992. Resistance of indigenous and cross-bred goats to heartwater (*Cowdria ruminantium*). *Recent Advances in Goat Production. (Proceedings of the fifth International Conference on Goats)*, New Delhi, India, 2–8 March. 1992: 1716–1719.
- DU PLESSIS, J.L. 1981. The application of the indirect fluorescent antibody test to the serology of heartwater. in: *Proceedings of an International Conference on Tick Biology and Control, Rhodes University, Grahamstown, Republic of South Africa*, edited by G.B. Whitehead & J.P. Gibson: 47–52.
- DU PLESSIS, J.L. & KÜMM, N.A.L. 1971. The passage of *Cowdria ruminantium* in mice. *Journal of the South African Veterinary Medical Association*, 42: 217–221.
- DU PLESSIS, J.L. & MALAN, L. 1987a. The application of the indirect fluorescent antibody test in research on heartwater. *Onderstepoort Journal of Veterinary Research*, 54: 319–325.
- DU PLESSIS, J.L. & MALAN, L. 1987b. The block method of vaccination against heartwater. *Onderstepoort Journal of Veterinary Research*, 54: 493–495.
- DU PLESSIS, J.L., JANSEN, B.C. & PROZESKY, L. 1983. Heartwater in Angora goats. I. Immunity subsequent to infection and treatment. *Onderstepoort Journal of Veterinary Research*, 50: 137–153.
- DU PLESSIS, J.L., VAN GAS, L., OLIVIER, J.A. & BEZUIDENHOUT, J.D. 1989. The heterogeneity of *Cowdria ruminantium* stocks: cross-immunity and serology in sheep and pathogenicity to mice. *Onderstepoort Journal of Veterinary Research*, 56: 195–201.
- DU PLESSIS, J.L., BEZUIDENHOUT, J.D., BRETT, M.S., CAMUS, E., JONGEJAN, F., MAHAN, S.M. & MARTINEZ, D. 1993. The sero-diagnosis of heartwater: a comparison of 5 tests. *Revue d'Élevage et de Médecine Vétérinaire des Pays Tropicaux*, 46: 123–129.
- DU PLESSIS, J.L., DE WAAL, D.T. & STOLTSZ, W.H. 1994. A survey of the incidence and importance of the tick-borne diseases heartwater, redwater and anaplasmosis in the heartwater endemic regions of South Africa. *Onderstepoort Journal of Veterinary Research*, 61: 295–301.
- EDINGTON, A. 1898. Heartwater. *Agricultural Journal of the Cape of Good Hope*, 12: 749–754.
- FULLER, C. 1924. Tsetse in the Transvaal and surrounding territories. An historical review. *9th & 10th Reports of the Director of Veterinary Educational Research*, Department of Agriculture, April 1923: 315–379.
- GRUSS, B. 1983. The danger of immunizing Boer goats against heartwater. *Journal of the South African Veterinary Medical Association*, 54: 67–68.
- HOWELL, C.J., DE VOS, A.J., BEZUIDENHOUT, J.D., POTGIETER, F.T. & BARROWMAN, P.R. 1981. The role of chemical tick eradication in the control or prevention of tick-transmitted diseases of cattle, in *Proceedings of an International Conference on Tick Biology and Control, Rhodes University, Grahamstown, Republic of South Africa*, edited by G.B. Whitehead & J.D. Gibson: 61–66.
- HUTCHEON, D. 1900. The history of heartwater. *Agricultural Journal of the Cape of Good Hope*, 17: 410–417.
- IMMELMAN, A. & DREYER, G. 1982. The use of doxycycline to control heartwater in sheep. *Journal of the South African Veterinary Association*, 53: 23–24.
- JONGEJAN, F., THIELEMANS, M.J.C., BRIERE, C. & UILENBERG, G. 1991a. Antigenic diversity of *Cowdria ruminantium* isolates determined by cross-immunity. *Research in Veterinary Science*, 44: 186–189.
- JONGEJAN, F., THIELEMANS, M.J.C., VAN KOOTEN, P.J.S., DE GROOT, M. & 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. *Veterinary Microbiology*, 28: 199–211.
- JONGEJAN, F. 1992a. Experimental transmission of *Cowdria ruminantium* (Rickettsiales) by the American reptile tick *Amblyomma dissimile* Koch, 1844. *Experimental Applied Acarology*, 15: 117–121.
- JONGEJAN, F. 1992b. Serodiagnosis of *Cowdria ruminantium*: current status. *Recent Developments in the Control of Anaplasmosis, Babesiosis and Cowdriosis*. Publication: ILRAD, Nairobi: 67–77.
- JONGEJAN, F., VAN VLIET, A.H.M. & VAN DER ZEIST, B.A.M. 1994. Molecular cloning and expression of the gene encoding the immunodominant 32 kilodalton protein of *Cowdria ruminantium*. *Proceedings of a FAO Expert Consultancy, Use of Applicable Biotechnological Methods for Diagnosing Haemoparasites, Merida, Mexico, 4–6 October 1993* (in press).
- LAWRENCE, J.A., WHITELAND, A.P., MALIKA, J., KAFUWA, P.T. & JONGEJAN, F. 1993. Use of serological response to evaluate heartwater immunization of cattle. *Revue d'Élevage et de Médecine Vétérinaire des Pays Tropicaux*, 46: 211–215.
- LOGAN, L.L., HOLLAND, C.J., MEBUS, C.A. & RISTIC, M. 1986. Letters: Serological relationship between *Cowdria ruminantium* and certain ehrlichia. *Veterinary Record*, 119: 458–459.
- LOGAN, L.L., WHYARD, T.C., QUINTERO, J.C. & MEBUS, C.A. 1987. The development of *Cowdria ruminantium* in neutrophils. *Onderstepoort Journal of Veterinary Research*, 54: 197–204.
- LOGAN, L.L., BIRNIE, E.F. & MEBUS, C.A. 1987. Cross-immunity between isolates of *Cowdria ruminantium*. *Onderstepoort Journal of Veterinary Research*, 54: 345 (Abstract only).
- LOUNSBURY, C.P. 1900. Tick heartwater experiments. *Agricultural Journal of the Cape of Good Hope*, 16: 682–687.
- LOUNSBURY, C.P. 1902. Heartwater in calves. *Agricultural Journal of the Cape of Good Hope*, 21: 165–169.
- LOUNSBURY, C.P. & ROBERTSON, W. 1904. Persian sheep and heartwater. *Agricultural Journal of the Cape of Good Hope*, 25: 175–186.
- MACKENZIE, P.K.I. & MCHARDY, N. 1987. *Cowdria ruminantium* infection in the mouse: a review. *Onderstepoort Journal of Veterinary Research*, 54: 257–269.
- MAHAN, S.M., WAGHELA, S.D., MCGUIRE, T.C., RURANGIRWA, F.R., WASSINK, L.A. & BARBET, A.F. 1992a. A cloned DNA probe for *Cowdria ruminantium* hybridizes with eight heartwater strains and detects infected sheep. *Journal of Clinical Microbiology*, 30: 981–986.
- MAHAN, S.M., WAGHELA, S.D., RURANGIRWA, F.R., YUNKER, C.E., CRAWFORD, T.B., BARBET, A.F., BURRIDGE, M.J. & MCGUIRE, T.C. 1992b. DNA probes for *Cowdria ruminantium*. *Recent Developments in the Control of Anaplasmosis, Babesiosis and Cowdriosis*. Publication: ILRAD, Nairobi: 79–83.
- MAHAN, S.M., MCGUIRE, T.C., JONGEJAN, F., RURANGIRWA, F.R. & BARBET, A.F. 1993a. Molecular cloning of a *Cowdria ruminantium* gene specific for an immunodominant 21 Kd protein. Abstract. 2nd Biennial Meeting of the American Society for Tropical Veterinary Medicine, Guadeloupe, West Indies, 2–6 February: 23.
- MAHAN, S.M., TEBELE, N., MUKWEDEYA, D., SEMU, S., NYATHI, C.B., WASSINK, L.A., KELLEY, P.J., PETER, T. & BARBET, A.F. 1993b. An immunoblotting diagnostic assay for heartwater based on the immunodominant 32-kilodalton protein of *Cowdria ruminantium* detects false positives in field sera. *Journal of Clinical Microbiology*, 31: 2729–2737.
- MAMMERICKX, M. 1961. Les buffles domestiques d'Asie importés au Congo. *I.N.E.A.C. Série technique*, 24: 50–51.
- MARÉ, C.J. 1984. *Heartwater. Foreign Animal Diseases. Their Prevention, Diagnosis and Control*. Publication United States Animal Health Association, Richmond, VA, USA: 186–194.

- MARTINEZ, D., SWINKELS, J.T., CAMUS, E. & JONGEJAN, F. 1990. Comparaison de trois antigènes pour le sérodiagnostique de la cowdriose. *Revue Élevage et de Médecine Vétérinaire des Pays Tropicaux*, 43:159–166.
- MATHERON, G., BARRÉ, N., CAMUS, E. & GOGUE, J. 1987. Genetic resistance of Guadeloupe native goats to heartwater. *Onderstepoort Journal of Veterinary Research*, 54:337–340.
- MULLER KOBOLD, A., MARTINEZ, D., CAMUS, E. & 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*. *Journal of Clinical Microbiology*, 30:1870–1873.
- NEITZ, W.O. 1940. Uleron in the treatment of heartwater. *Journal of the South African Veterinary Medical Association*, 11:15.
- NEITZ, W.O. & ALEXANDER, R.A. 1941. The immunization of calves against heartwater. *Journal of the South African Veterinary Medical Association*, 12:103–111.
- NEITZ, W.O. & ALEXANDER, R.A. 1945. Immunization of cattle against heartwater and the control of the tick-borne diseases, redwater, gallsickness and heartwater. *Onderstepoort Journal of Veterinary Science and Animal Industry*, 20:137–158.
- NEITZ, W.O. 1947. Die oordraging van hartwater deur *Amblyomma pomposum* Dönitz 1909. *South African Journal of Science*, 1: 83.
- NEITZ, W.O. 1968. Heartwater. *Bulletin. Office International des Epizooties*, 70: 329–336.
- NORVAL, R.A.I., ANDREW, H.R. & YUNKER, C.E. 1990. Infection rates with *Cowdria ruminantium* of nymphs and adults of the bont tick *Amblyomma hebraeum* collected in the field in Zimbabwe. *Veterinary Parasitology*, 36:277–283.
- NORVAL, R.A.I., ANDREW, H.R., YUNKER, C.E. & BURRIDGE, M.J. 1992. Biological processes in the epidemiology of heartwater, in *Tick Vector Biology. Medical and Veterinary Aspects*, edited by B. Fivaz, T. Petney & I. Horak. New York: Springer-Verlag: 71–86.
- OBEREM, P.T. & BEZUIDENHOUT, J.D. 1987. Heartwater in hosts other than domestic ruminants. *Onderstepoort Journal of Veterinary Research*, 54:271–275.
- PEPIN, L., CAMUS, E., MATHERON, G. & BENSALD, A. 1993. Use of microsatellites as genomic markers to study resistance to cowdriosis. *Revue d'Élevage et de Médecine Vétérinaire des Pays Tropicaux*, 46:209 (Abstract only).
- PERREAU, P., MOREL, P.C., BARRÉ, N. & DURAND, P. 1980. Existence de la cowdriose (heartwater) à *Cowdria ruminantium* chez la petit ruminants des Antilles françaises (la Guadeloupe) et des Mascareignes (la Reunion et l'île Maurice). *Revue d'Élevage et de Médecine Vétérinaire des Pays Tropicaux*, 33: 21–22.
- PETNEY, T.N., HORAK, I.G. & RECHAV, Y. 1987. The ecology of the African vectors of heartwater, with particular reference to *Amblyomma hebraeum* and *Amblyomma variegatum*. *Onderstepoort Journal of Veterinary Research*, 54:381–395.
- PIENAAR, J.G., BASSON, P.A. & VAN DER MERWE, J.L. DEB. 1966. Studies on the pathology of heartwater [*Cowdria (Rickettsia) ruminantium* (Cowdry 1926)]. I. Neuropathological changes. *Onderstepoort Journal of Veterinary Research*, 33: 115–138.
- POISSON, H. & GEOFFROY () 1925. [Cited by Provost, A. and Bezuidenhout, J.D. 1987, *vide* Curasson, G. 1936. Heartwater. *Traité de pathologie exotique vétérinaire comparée*. Paris: Vigot, 2:316–353.]
- PRELLER, G.S. 1938. *Dagboek van Louis Trichardt (1836–1838). Met inleiding en aantekeninge*. Cape Town: Nasionale Pers: 273–274; 288–289.
- PROVOST, A. & BEZUIDENHOUT, J.D. 1987. The historical background and global importance of heartwater. *Onderstepoort Journal of Veterinary Research*, 54:165–169.
- PROZESKY, L. 1987. The pathology of heartwater. III. A review. *Onderstepoort Journal of Veterinary Research*, 54:281–286.
- PURCHASE, H.S. 1945. A simple and rapid method for demonstrating *Rickettsia ruminantium* (Cowdry 1925) in heartwater brains. *Veterinary Record*, 57:413–414.
- PURNELL, R.E. 1984. Control of heartwater in cattle in southern Africa using Terramycin/LA. *Preventive Veterinary Medicine*, 2: 239–254.
- PURNELL, R.E. 1987. Development of a prophylactic regime using terramycin/LA to assist in the introduction of susceptible cattle into heartwater endemic areas of Africa. 54:509–512.
- RISTIC, M. & HUXSOLL, D.L. 1984. Tribe II. Ehrlichieae Philip 1957, 948, in *Bergey's Manual of Systematic Bacteriology*, edited by N.R. Krieg & J.G. Holt. Baltimore: Williams & Wilkins, 1:704–711.
- RUFF, G., MAILLARD, J.C., CAMUS, E., DEPRES, E. & MATHERON, G. 1993. Occurrence of caprine leucocyte antigens (CLA) in Creole goats susceptible/resistant to heartwater. *Revue d'Élevage et de Médecine Vétérinaire des Pays Tropicaux*, 46: 205–207.
- SCOTT, G.R. 1987. The taxonomic status of the causative agent of heartwater. *Onderstepoort Journal of Veterinary Research*, 54:257–260.
- SEMU, S., MAHAN, S.M., YUNKER, C.E. & BURRIDGE, M.J. 1992. Development and persistence of *Cowdria ruminantium* antibodies following experimental infection of cattle, as detected by the indirect fluorescent antibody test. *Veterinary Immunology and Immunopathology*, 33:339–352.
- SOLDAN, A.W., NORMAN, T.L., MASAKA, S., PAXTON, E.A., EDELSTEN, R.M. & SUMPTION, K.J. 1993. Seroconversion to *Cowdria ruminantium* of Malawi zebu calves, reared under different tick control strategies. *Revue d'Élevage et de Médecine Vétérinaire des Pays Tropicaux*, 46:171–177.
- SPREULL, J. 1922. Heartwater. *Agricultural Journal of the Union of South Africa*, 4: 236–255.
- STECK, W. 1928. Pathological studies on heartwater. *13th & 14th Reports of the Director of Veterinary Education and Research*, Department of Agriculture, October: 283–309.
- STILLER, D. 1990. Application of biotechnology for the diagnosis and control of ticks and tickborne diseases. *Parassitologia*, 32:87–111.
- SYNGE, B.A. 1978. Brain biopsy in the diagnosis of heartwater. *Tropical Animal Health and Production*, 10:45–48.
- THOMAS, A.D. & MANSVELT, P.R. 1957. The immunization of goats against heartwater. *Journal of the South African Veterinary Medical Association*, 28:163–168.
- UILENBERG, G. 1971a. Études sur la cowdriose à Madagascar. Première partie. *Revue d'Élevage et de Médecine Vétérinaire des Pays Tropicaux*, 24:239–249.
- UILENBERG, G. 1971b. Études sur la cowdriose à Madagascar. Deuxième partie. *Revue d'Élevage et de Médecine Vétérinaire des Pays Tropicaux*, 24:355–364.
- UILENBERG, G. 1981. Heartwater disease in Diseases of cattle in the tropics, edited by M. Ristic & I. McIntyre. The Hague, The Netherlands: Nijhoff Publication: 345–360.
- UILENBERG, G. 1982. Experimental transmission of *Cowdria ruminantium* by the Gulf Coast tick *Amblyomma maculatum*: danger of introducing heartwater and benign African theileriosis onto the American mainland. *American Journal of Veterinary Research*, 43:1279–1282.
- UILENBERG, G. 1983. Heartwater (*Cowdria ruminantium* infection): Current status. *Advances in Veterinary Science and Comparative Medicine*, 27:427–480.
- UILENBERG, G. 1989. La cowdriose caprine, in *African small ruminant research and development*, edited by R.T. Wilson & A.

- Melaku. African Small Ruminant Research Network: Addis Ababa: ILCA: 427–435.
- UILENBERG, G. 1990a. Extension de la tique *Amblyomma variegatum* dans les Antilles: comment expliquer cette grave menace et que faire? *Revue d'Élevage et de Médecine Vétérinaire des Pays Tropicaux*, 43:297–299.
- UILENBERG, G. 1990b. Methods currently used for the control of heartwater: Their validity and proposals for future control strategies. *Parassitologia*, 32: 55–62.
- UILENBERG, G. 1994. Recent advances in the diagnosis of heartwater. *Proceedings of a FAO Expert Consultancy, Use of Applicable Biotechnological Methods for Diagnosing Haemoparasites. Merida, Mexico, 4–6 October 1993* (in press).
- VAN AMSTEL, S.R. & OBEREM, P.T. 1987. The treatment of heartwater. *Onderstepoort Journal of Veterinary Research*, 54:475–479.
- VAN DE PYPEKAMP, H. & PROZESKY, L. 1987. Heartwater. An overview of the clinical signs, susceptibility and differential diagnoses of the disease in domestic ruminants. *Onderstepoort Journal of Veterinary Research*, 54:263–266.
- VAN VLIET, A.H., JONGEJAN, F. & VAN DER ZEIJST, B.A. 1992. Phylogenetic position of *Cowdria ruminantium* (Rickettsiales) determined by analysis of amplified 16S ribosomal DNA sequences. *International Journal of Systematic Bacteriology*, 42: 494–498.
- WAGHELA, S.D., RURANGIRWA, F.R., MAHAN, S.M., YUNKER, C.E., CRAWFORD, T.G., BARBET, A.F., BURRIDGE, M.J. & McGUIRE, T.C. 1991. A cloned DNA probe identifies *Cowdria ruminantium* in *Amblyomma variegatum* ticks. *Journal of Clinical Microbiology*, 29:2571–2577.
- WALKER, J.B. & OLWAGE, A. 1987. The tick vectors of *Cowdria ruminantium* (Ixodoidea, Ixodidae, genus *Amblyomma*) and their distribution. *Onderstepoort Journal of Veterinary Research*, 54: 353–379.
- WHITE, F. 1983. *The Vegetation of Africa*. Paris: UNESCO.
- YUNKER, C.E., BYROM, B. & SEMU, S. 1988. Cultivation of *Cowdria ruminantium* in bovine vascular endothelial cells. *Kenya Veterinarian*, 12:12–16.
- YUNKER, C.E. 1991. The epidemiology, diagnosis and control of heartwater, in *Cowdriosis and Dermatophilosis of Livestock in the Caribbean Region*. Trinidad and Tobago: Caribbean Agricultural Research Development Institute, St. Augustine: 87–96.
- YUNKER, C.E., MAHAN, S.M., WAGHELA, S.D., McGUIRE, T.C., RURANGIRWA, F.R., BARBET, A.F. & 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. *Epidemiology and Infection*, 110:95–104.
- YUNKER, C.E. & ALLSOPP, B.A. 1994. Modern biotechnological methods for diagnosis of cowdriosis—present and future trends. *Proceedings of a FAO Expert Consultancy, Use of Applicable Biotechnological Methods for Diagnosing Haemoparasites, Merida, Mexico, 4–6 October 1993*. FAO Publication, Rome, Italy: 143–155.