Heartwater

Disease Monograph Series – 11

Bacteria | *Ehrlichia ruminantium* | Cattle | Sheep | Goats | Wild Ruminants

IDRC | Bartay

This monograph forms part of a series of disease monographs commissioned by the International Development Research Centre over the period Nov 2015 to April 2016 to inform funding priorities for the Livestock Vaccine Innovation Fund (LVIF). The LVIF is a seven-and-a-half year, CA$57 million partnership between the Bill & Melinda Gates Foundation, Global Affairs Canada and Canada's International Development Research Centre. It focuses on those animal diseases posing the greatest risk to poor livestock keepers in Sub-Saharan Africa, South and Southeast Asia, targeting transboundary diseases to achieve lasting regional impact.

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Acronyms

AU	African Union
AU-IBAR	African Union Inter-African Bureau for Animal Resources
BBRSC	Biotechnology and Biological Sciences Research Council
CVO	Chief Veterinary Officer
DIVA	Differentiate infected from vaccinated animals
DVS	Director Veterinary Services
ELISA	Enzyme-linked immunosorbent assay
FAO	Food and Agriculture Organization of the United Nations
IAEA	International Atomic Energy Agency of the United Nations
IM	Intramuscular
NGO	Non-governmental organization
OIE	World Animal Health Organization
OVI	Onderstepoort Veterinary Institute
OBP	Onderstepoort Biological Products
PCR	Polymerase chain reaction
SHF	Small holder farmer
SMP-AH	Standard Methods and Procedures in Animal health Program
TBD	Tick borne diseases

TPP	Target Product Profile
WHO	World Health Organization of the United Nations

Executive Summary

Etiology and relevance

Heartwater or cowdriosis is specific to cattle, sheep, goats and some wild ruminants, and is prevalent in much of Africa and the Caribbean. It results from infection by *Ehrlichia* (formerly *Cowdria*) *ruminantium*, a small, Gram negative, pleomorphic coccus in the family *Anaplasmataceae* and order Rickettsiales. This organism is an obligate intracellular parasite. Strains of *E*. *ruminantium* are very diverse; while some strains are highly virulent, others appear to be non-pathogenic. *E. ruminantium* has a high level of genomic plasticity. Gene segments are often deleted or inserted, and genes may be disrupted. Several different genotypes can co-exist in a geographic area, and may recombine to form new strains

E. *ruminantium* is transmitted by ticks of the genus *Amblyomma*.

Heartwater is most severe in small ruminants, but also causes heavy losses in exotic cattle, which are more susceptible than indigenous breeds. However, indigenous cattle can also be affected if poor conditions weaken their immune system, or if animals are moved from an area free of heartwater to an area in which it is endemic.

Heartwater is difficult to diagnose and only Giemsa-stained smears of crushed cerebral grey matter can confirm the presence of colonies of the organism in the endothelial cells of the capillaries. Small ruminants affected by *Cowdria* present distinctive nervous symptoms: in peracute forms, animals generally drop suddenly to the ground, start 'pedalling' with their legs and rapidly die. These nervous symptoms are very common in the later stages of the disease, but can be confused with signs of poisoning or other diseases such as tetanus or rabies. Postmortems are rarely performed and accurate information on the incidence of heartwater is generally lacking. Furthermore, serological tests are generally not satisfactory due to cross reactions with other organisms such as *Ehrlichia*.

In tropical and subtropical areas, the disease is endemic and results in considerable economic losses due to loss of production, treatment costs and reduced initiatives for the upgrading of local breeds of livestock with more susceptible exotic breeds. Heartwater is considered by some groups as the second most economically important tick borne disease of livestock in Africa, after ECF. Furthermore, it affects not only cattle, but essentially small ruminants, considered to be of great importance to poor livestock keepers. Prevalence information is not always available and in some studies is shown to be low. This is attributed to endemic stability in affected areas. The endemic situation however restricts the introduction of improved naïve breed or animals in these affected regions.

Control and immunisation

Heartwater has traditionally been controlled by the use of chemical acaricides to prevent or reduce transmission by *Amblyomma spp*. Oxytetracycline treatment is normally successful if administered early, and is also used in a prophylactic manner on very valuable and susceptible animals during the peak *Amblyomma* season. This

method, although not recommended by veterinarians, is widely used by commercial farmers in South Africa and by local farmers when they have to move their cattle through an endemic area. The protection conferred by the antibiotic is effective not only against infection with heartwater, but also against other tick-borne diseases, and animals may develop their own immunity if naturally challenged during this coverage.

A blood-based vaccine containing live Cowdria organisms is used in South Africa. It is an "infection and treatment" type of immunization using live organisms generated from blood of live sheep used for vaccination of animals that are subsequently treated with antibiotics at specific time during the course of infection. This blood vaccine is the only registered vaccine in the South Africa and nothing in any other countries. The presence of wide diversity in stocks of *E ruminantium* genotypes circulating in animals and ticks in different geographical regions resulting in different immunogenic types hamper vaccine development and limit the wide use of the current commercial blood vaccine. It does not protect against all of the isolates and, therefore, not used beyond South Africa, and even within South Africa, not throughout the disease endemic areas. Furthermore the vaccine requires a strict cold chain and can cause severe clinical reactions.

Over the years, research has focused on inactivated vaccine, with two groups having worked extensively on the development work: the University of Florida and CIRAD. These two institutions have inactivated different stocks of the *E*. *ruminantium* organism, as single or as combination of stocks. Despite good results obtained on small scale and generally homologous challenge studies, these early successes were unfortunately not repeated when trials were conducted in heterologous challenge studies or in field situations, where natural tick challenge with genotypes having differing immunogenicities would have occurred. Several reports indicate that under these circumstances the vaccine reduces mortality levels, but protection levels have been disappointing. The different studies conducted by the University of Florida group for example show that overall mortality levels of 71% in naive animals can be reduced to 36% by vaccination.

A number of positive outcomes have however been achieved which could contribute to the development of better vaccine for Heartwater. These include the development of efficient and cost effective cell culturing system, better inactivation processes and identification of suitable adjuvants. The CIRAD group has been able to demonstrate a drop in the production cost of a potential inactivated Heartwater vaccine to around US$0.14 per dose.

Having successfully managed to attenuate the cross-protective Welgevonden stock and developed a cell culture system, the OVI has developed an attenuated vaccine that is currently undergoing field evaluation in South Africa.

Recombinant technology has been used to develop DNA vaccines formulations which have been tried in mice and sheep: most of the work has been based on the immunisation with DNA vaccine containing the *map1* gene of *E. ruminantium.* None of the different attempts or forms has been taken beyond proof of concept

The future if Heartwater vaccines and vaccination

Given the importance of the disease, and also the fact that it affects most poor livestock keepers in Sub-Saharan Africa who keep small ruminants, and also given the challenges associated with chemotherapy and vector control through the use of acaricides, immunisation remains the best options for the control of this important disease. A number of actions could be considered in order to contribute toward the development of effective immunisation approaches:

- There is a need to validate molecular tools for rapid field strain matching to possible vaccines
- Evaluate inactivated form of vaccines based on broad spectrum isolates or stock, such as Welgevonden, using knowledge accumulated by the different groups
- Evaluate vaccines based on mixed stocks, live attenuated or inactivated
- Evaluate combination vaccination program, with priming with inactivated vaccine and boosting with live attenuated vaccine
- Conduct a full development on the attenuated Welgevonden vaccine, essentially assess reversion to virulence and possibly lyophilisation
- Development of robust challenge models, which should preferably include a tick challenge
- Conduct a detailed assessment of recombinant candidate vaccines, and include vaccine development knowledge to the groups currently developing these approaches, which generally are made of scientists with no vaccine development expertise

Clinical disease overview

Etiology

The first reference to what may have been heartwater was made in South Africa by the Voortrekker pioneer Louis Trichardt in 1838 [32]. It is only in 1900 that Lounsbury published his confirmation of the long-standing suspicion that the bont tick (*Amblyomma hebraeum*) was the vector of heartwater in South Africa, but another quarter of a century elapsed before Cowdry demonstrated that the infectious agent in the tissues of infected animals and ticks was a rickettsia which he named Rickettsia ruminantium. The name was later changed to Cowdria ruminantium and eventually to *Ehrlichia ruminantium* [3]

Heartwater results from infection by *Ehrlichia ruminantium*, a small, Gram negative, pleomorphic coccus in the family *Anaplasmataceae* and order *Rickettsiales* (Figure 1). This organism is an obligate intracellular parasite. Strains of E. *ruminantium* are very diverse; while some strains are highly virulent, others appear to be non-pathogenic. *E*. *ruminantium* has a high level of genomic plasticity. Gene segments are often deleted or inserted, and genes may be disrupted. Several different genotypes can co-exist in a geographic area, and may recombine to form new strains.

E. ruminantium was traditionally classified as the sole species of the genus Cowdria, tribe Ehrlichieae, family Rickettsiaceae, order Rickettsiales [33]. It had long been realized, however, that the organism had a close antigenic relationship with certain *Ehrlichia spp*., and in 1992 the first molecular phylogeny of the organism, based on the 16S ribosomal RNA gene sequence of the Zimbabwean Crystal Springs isolate, revealed that it was related to *Anaplasma marginale*. This was closely followed by an analysis of the (slightly different) 16S sequence of the Senegal isolate, which showed a closer relationship to *Ehrlichia canis* and *Ehrlichia chaffeensis* than to *E. ruminantium* [11].

As the 16S genes of more organisms of the family Rickettsiaceae were sequenced it became evident that the genus Ehrlichia did not constitute a monophyletic group, and that organisms classified as Anaplasma, Cowdria, and Neorickettsia were members of three separate clades, each of which included various organisms classified as *Ehrlichia spp*. [22]. These clades became known as genogroups I, II and III Ehrlichia (Figure 2) with *C. ruminantium* being included in genogroup III, together with *E. canis*, *E. chaffeensis, E. muris* and *E. ewingi*. [3]. See Figure 2.

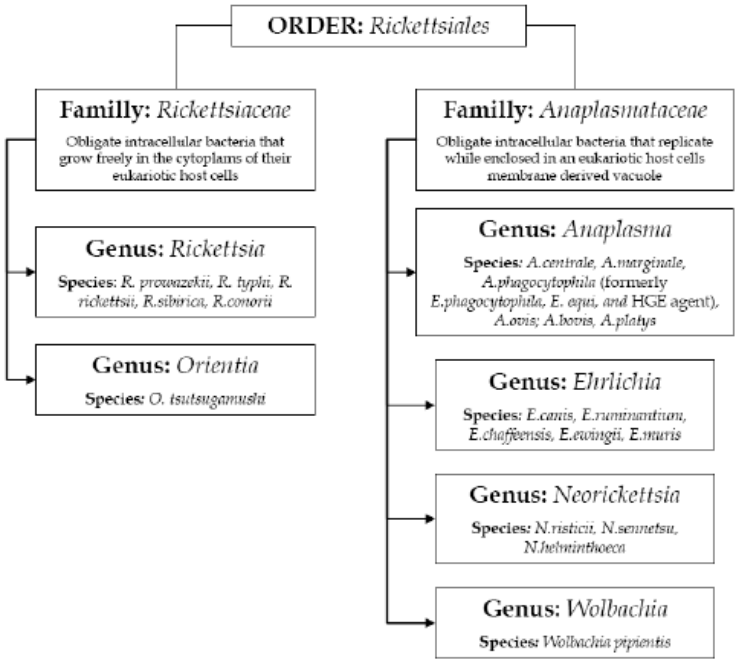

Figure 1: Classification of Ehrlichia within the order Rickettsiales

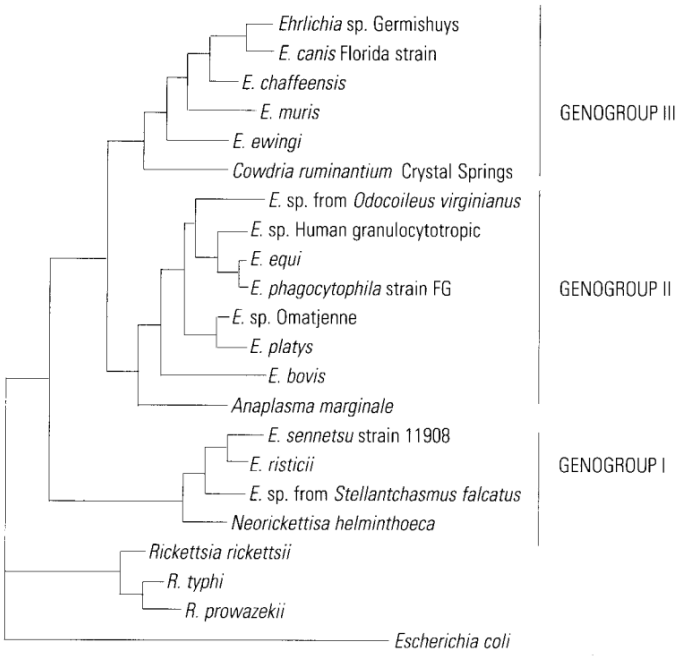

Figure 2: Maximum likelihood tree based on 16S ribosomal RNA gene sequences in the order Rickettsiales before reclassification, with *Escherichia coli* as outgroup. Note species classified as *Ehrlichia* occurring in three separate paraphyletic clades (genogroups). *Cowdria ruminantium* is located in genogroup III [3]

Genetic diversity and poor cross protective ability among genotypes or stocks

As the 16S genes of more isolates of *Cowdria* became available it was found that *E. ruminantium* was not a relatively homogeneous entity and that there were several distinct Cowdria 16S genotypes, all of which fell into a tight cluster within the genogroup III *Ehrlichia*. It is now known that there is far more genetic variability among *E*. *ruminantium* organisms than had ever been suspected [11][22].

Based on existing stocks of *E*. *ruminantium*, 8 different 16S ribosomal RNA genotypes are known, all classified at present as *E*. *ruminantium*. However, 16S sequence data cannot be used as the sole determinant of what constitutes a species; it is also important to be able to grow a stock in tissue culture in order to accurately determine its disease-causing status, and this has not been done for several of these eight genotypes. Some of them are undoubtedly justifiably classified as *E*. *ruminantium*, but others may need to be reclassified in the future in the light of their infectivity and pathogenicity in ruminant hosts [3]. See Table 1.

Table 1: Details of several well-characterized *E. ruminantium* stocks currently being used for research [3]

ISOLATE	REMARKS	ORIGIN	16S GENOTYPE
Ball 3	S. African blood 'vaccine' isolate	Natural field infection, mammalian host not specified, Limpopo Province, S. Africa	Ball 3
Blaauwkrans	Highly pathogenic to goats in Eastern Cape	*A. hebraeum* tick from an eland, Eastern Cape, South Africa	Not determined
Crystal Springs	–	Zimbabwe	Crystal Springs
Gardel	Common Caribbean genotype	Guadeloupe	Gardel
Kümm 1	Two genotypes obtained from a single isolate	Naturally infected goat, Northern Province, South Africa	Senegal
Kümm 2			Omatjenne
Kwanyanga	Not highly pathogenic to sheep	Naturally infected sheep in the Eastern Cape, South Africa	Crystal Springs
Mara 87/7	Widespread South African genotype	*A. hebraeum* tick, Northern Province, South Africa	Mara 87/7
Senegal	Attenuates readily in culture	West Africa	Senegal
Welgevonden	Type specimen *E. ruminantium* comb. nov.	South Africa	Crystal Springs

With the now well recognised existence of immunogenic variants, it is obviously of crucial importance for the development of vaccines to have an understanding of which stocks can confer complete or partial cross-immunity one to another in ruminants. Poor cross-protection between stocks or even none at all, has been shown in several experiments but several of these experiments included at least one stock now known to be genetically heterogeneous. Table 2 below provides the result of an experiment carried out at Onderstepoort with six different *E. ruminantium* stocks in sheep [3]. Full cross-protection was essentially seen only with the Welgevonden stock.

Table 2: Cross-immunity protection engendered in sheep between various *E. ruminantium* isolates [3]

STOCK TO WHICH IMMUNE	CHALLENGE STOCK					
	WELGEVONDEN	BALL 3	GARDEL	MARA 87/7	KWANYANGA	BLAAUWKRANS
Welgevonden	+	+	+	+	+	+
Ball 3	–	+	–	+/–	+	+/–
Gardel	–	+/–	+	–	+/–	–
Mara 87/7	–	+	–	+	+/–	+/–
Kwanyanga	–	+/–	+/–	–	+	–
Blaauwkrans	–	+/–	–	+/–	–	+

\+ complete cross-protection
– no cross-protection
+/– partial cross-protection

Table 3: Variability among *E. ruminantium* stocks as shown in Infectivity studies: three different types of pathogenicity are evidenced [5]

Genotype	Origin		Pathogenicity		
	Geographical	Biological	Cattle	Sheep and goats	Mice
Ball 3	South Africa	Bovine	+	+	-
Gardel	Guadeloupe	*A. hebraeum*	+	+	0
Kiswani	Kenya	Bovine	+	+	ND
Mara 87/7	South Africa	*A. hebraeum*	+	+	+
Omatjenne	Namibia	*H. truncatum*	-	+	-
Senegal	Senegal	Bovine	+	+ /-	+ /-
Welgevonden	South Africa	*A. hebraeum*	+	+	+

+=pathogenic; +/-=mildly pathogenic; -= non-pathogenic; ()= non-infective; ND= not done

Epidemiology

Susceptible animal species

Domestic ruminants, notably cattle, sheep and goats are most susceptible to heartwater. Heartwater is most severe in small ruminants, but also causes heavy losses in exotic cattle, which are more susceptible than indigenous breeds. As shown in Table 4 below, 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 [21]. 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.

Table 4: The susceptibility of various wild ruminants to *E. ruminantium* (adapted from [21])

HOSTS (AFRICAN)		SUSCEPTIBILITY		
COMMON NAME	SCIENTIFIC NAME	CLINICAL	SUB-CLINICAL	REFRACTORY
Giraffe	*Giraffa camelopardalis*		+	+
Black wildebeest	*Connochaetes gnu*	+	+	+
Blue wildebeest	*Connochaetes taurinus*			+
Red hartebeest	*Alcelaphus buselaphus*			+
Blesbok	*Damaliscus dorcas phillipsi*	+	+	
Duiker	*Cephalophus* sp.			+
Springbok	*Antidorcas marsupialis*	+		
Scimitar-horned oryx	*Oryx dammah*			+
African buffalo	*Syncerus caffer*		+	
Bushbuck	*Tragelaphus scriptus*			+
Eland	*Taurotragus oryx*	+	+	
HOSTS (NON-AFRICAN)				
White-tailed deer	*Odocoileus virginianus*	+		
Fallow deer	*Cervus dama*	+		
Timor deer	*Cervus timorensis*	+		
Water buffalo	*Bubalus bubalis*	+		
Barbary sheep	*Ammotragus lervia*	+		
Himalayan tahr	*Hemitragus jemlahicus*	+		
Nilgai	*Boselaphus tragocamelus*	+		
Blackbuck	*Antilope cervicapra*	+		
Mouflon	*Ovis orientalis*	+		

Vectors of Bovine anaplasmosis and transmission

Heartwater is transmitted by ticks from the genus Amblyomma. Ticks become infected as larvae or nymphs, and can transmit the disease as nymphs or adults. Transovarial passage is not significant in the epidemiology of heartwater, and may not occur. Cattle importations have been implicated in the dispersal of Amblyomma ticks into the Caribbean. At least twelve species of Amblyomma can transmit E. *ruminantium*. *A*. *variegatum* (the tropical bont tick) is the major vector in Africa and the Caribbean (Figure 3). Subsequently the distribution of the disease in Africa coincides with that of the two most important vector species, *A*. *variegatum* and *A*. *hebraeum,* the later, the bont tick, especially in southern Africa [31]. Other known vectors include *A*. *lepidum* (in East Africa and the Sudan), *A*. *astrion*, and *A*. *pomposum*. *A*. *sparsum*, *A*. *gemma*, *A*. *cohaerans*, *A*. *marmoreum* and *A*. *tholloni* (the elephant tick) are capable of transmitting experimental infections [31]. Two North American species, *A*. *maculatum* (the Gulf Coast tick) and *A*. *cajennense*, can transmit *E*. *ruminantium* in the laboratory, but neither has been implicated in natural infections. *E*. *ruminantium* gene segments have been found, by PCR, in the ticks *Rhipicephalus evertsi*, *Hyalomma truncatum* and *Hyalomma marginatum*; however, the organism was never isolated [24].

Ticks become infected by feeding on acutely ill or sub-clinically infected animals. Experimentally infected carrier sheep can infect ticks for at least seven months. Cattle can infect ticks for a minimum of eight months. Blesbok, black wildebeest, blue wildebeest, African buffalo, eland, giraffe, greater kudu and sable antelope can also become carriers [32]. Infections have been detected for up to six months in some wild ruminants. *E. ruminantium* is very fragile and does not survive outside a host for more than a few hours at room temperature.

However, cows may transmit the infection to their calves in colostrum [3].

Figure 3: *Amblyomma variegatum* (Tropical bont tick). [3]

Distribution

Heartwater is endemic in most of Africa south of the Sahara desert (Figure 4), as well as in surrounding islands such as Madagascar, and in the Caribbean.

The distribution of the disease in Africa coincides with that of the two most important vector species, *A. variegatum* and *A. hebraeum* [7]. The vector occurs in Africa, south of the Sahel area, from Senegal to Ethiopia and to the extreme north-west of Somalia. It is prevalent in central and eastern Africa, and also in part of Botswana, Zimbabwe and Mozambique. In eastern Angola, southern Democratic Republic of Congo (DRC) and in Zambia, it overlaps with *A. pomposum*; in Zimbabwe, it overlaps with A. hebraeum, and in East Africa (from Sudan to Tanzania) it is present simultaneously with *A. lepidum*. [31][33]

From its original source, *A. variegatum* was introduced in the Yemen, Arab republic and in different islands around Africa. It has thus been recorded in Capo Verde islands [31] and in Indian Ocean islands: Madagascar, La Réunion, Mauritius, the Seychelles and the four Comoros islands (Grand Comore, Anjouan, Moheli, Mayotte).

A. variegatum has also been introduced in the West Indies.

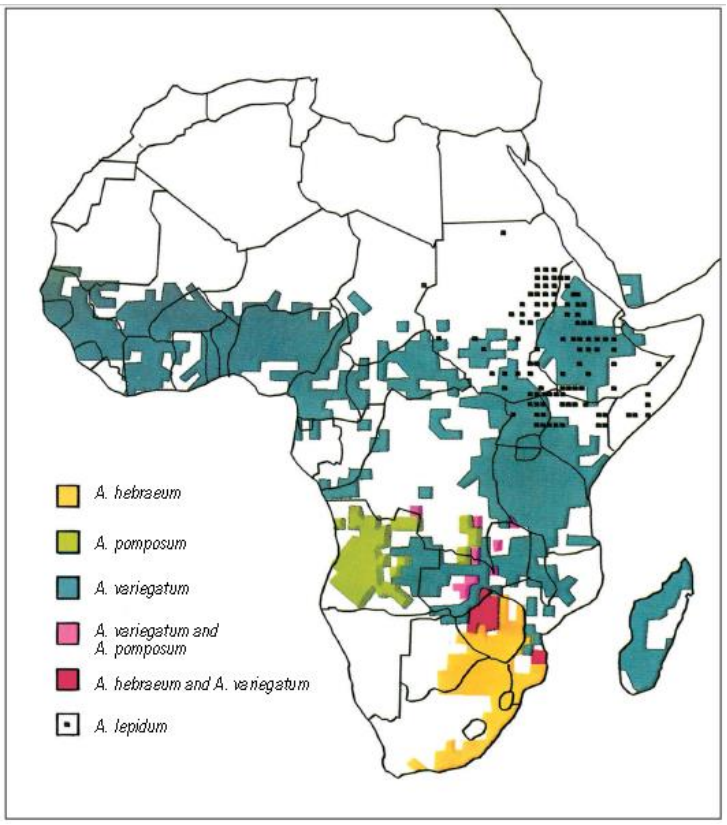

Figure 4: Distribution of the major Amblyomma spp. vectors of Ehrlichia ruminantium in Africa. [3]

Pathogenesis of Heartwater

Although heartwater pathogenesis is poorly understood despite being extensively studied, it is generally accepted that the determining lesion in the pathogenesis of heartwater is an increased vascular permeability of smaller blood vessels [12]. However, exactly how this increased permeability is caused is not yet clearly defined.

Van Amstel et al. [28] reviewed the clinical pathological changes that occur during a heartwater infection. Most of these changes coincide with the onset of the febrile reaction. A progressive anaemia develops during the course of infection and it has been suggested that this anaemia is caused by a bone marrow depression [28]. Together with a drop in haematocrit, a neutropaenia, an eosinopaenia and a lymphocytosis are the most marked and consistent changes seen in the haemogram associated with heartwater [28].

Immunity to Heartwater

Laboratory experiments as well as field observations have shown that cattle, sheep and goats are capable of developing a protective immunity against heartwater after surviving a virulent challenge (van Amstel, 1987). However, partial or total lack of cross protection between different isolates of E. ruminantium has been demonstrated (Du Plessis et al. 1994; Jongejan et al. 1991). Interestingly, immunity to the Welgevonden isolate of *E. ruminantium* has been shown to confer protection to a number of other virulent southern African stocks such as the Ball 3, Mara 87/7, Blaaukrans and Gardel [10][13] and it is for this reason that the Welgevonden stock would be the most suitable as a vaccine candidate for South Africa.

It is also known that young animals possess an innate resistance to the disease, irrespective of the immune status of the dam. In calves this lasts up to the age of four weeks and in lambs up to at least seven days [14].

The mechanism by which the immune response develops is not entirely clear, but it has been suggested that cellular immunity, rather than humoral immunity, is the predominant response [25][26]. This also became evident in a study by Mahan et al. [17] where they demonstrated that the inactivated vaccine prepared in adjuvants that preferentially induce humoral immunity did not protect against heartwater challenge, but vaccines prepared with adjuvants which induce cellular immunity were more efficient in protecting sheep against lethal heartwater challenge. Serum antibodies are produced in response to infection but they do not appear to correlate with protection or duration of immunity [5][26].

Clinical Signs

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.

Clinical signs in susceptible animals include fever, lack of appetite, incoordination, respiratory distress, nervous symptoms and death [23][32]. These signs are mostly related to an increased capillary permeability, which leads to the excess effusion of fluid into tissues and the body cavities [23]. The course of the disease can be quite variable, ranging from peracute to mild depending on the age, immune status, individual or breed susceptibility of the animal and virulence of the isolate [32].

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 [32]. The duration of this inverse-age resistance varies amongst species. It is reported to be 9 days in Merino lambs, 2 weeks in kids and 2 to 3 weeks in calves [9][32].

Subclinical infections: Mild or latent infections, revealed by a marked pyrexia and followed within 2-3 d by complete recovery, are known. The incidence of this form is unknown owing to the inapparent symptoms [23], 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 [32]. Recovered sheep, goats and cattle, as well as African buffalo, become long-term carriers [7].

Per-acute infections: This form of infection is characterized by sudden death some 36 h after clinical symptoms first appear. 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 peracute cases are more common in the former [3].

Acute infections: This is the classical, most common form of infection in susceptible animals [23]. Pyrexia of several days duration, subsiding when death approaches, is followed by listlessness, rapid pulse and tachypnoea, and a disturbed expression in the eyes. Nervous symptoms ensue, such as a twitching of facial muscles and high stepping gait [32]. 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 drop to subnormal, prior to death. Clinical signs may last 3- 5 days 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 [32]. 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.

Mortality rates vary between 5 % and 100 % [9][23]. Mortality rate in indigenous goats in an endemic area of Guadeloupe has been estimated at around 10 % [9].

Pathology: Most lesions are restricted to acute and sub-acute 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 liter 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 [9]. Splenomegaly occurs in over 90% sheep and goats, but enlargement may not be marked. Other workers have not frequently observed splenomegaly in small ruminants [9]. The lymph nodes are swollen and the kidneys somewhat pale. The liver is congested, often showing fatty degeneration, but hepatic lesions are not striking [9]. 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.

Diagnosis

Clinical Diagnosis

A presumptive or tentative diagnosis of heartwater is based on the presence of Amblyomma vectors, nervous signs, and presence of transudates in the pericardium and thorax on post-mortem examination.

In clinically ill animals, blood samples should be collected for PCR. PCR can sometimes detect organisms in the blood or bone marrow of carriers. For culture, blood is collected into an anticoagulant and diluted in culture medium; details are available in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals [33]. Samples should be kept refrigerated and shipped with ice packs. Serum may be collected for serology.

Differential Diagnosis

The peracute form of heartwater can be confused with anthrax. The acute form may resemble rabies, tetanus, bacterial meningitis or encephalitis, babesiosis, anaplasmosis, cerebral trypanosomiasis, or theileriosis. It must also be differentiated from poisoning with strychnine, lead, ionophores and other myocardial toxins, organophosphates, arsenic, chlorinated hydrocarbons, or some poisonous plants (*Cestrum laevigatum*, *Pavetta species*, and *Pachystigma species*). Accumulations of fluid similar to heartwater are also sometimes seen in heavy helminth infestations [9][23]

It is confirmed by the finding of characteristic colonies of the causal agent in capillary endothelial cells, particularly of brain smears [33], or by sub-inoculating 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 [32]; however, this is impractical on a routine basis for a number of reasons.

Pathology and post-mortem diagnosis

Post-mortem examinations are usually only conducted for particularly valuable animals, so, even in an endemic area, relatively few are carried out. The classical post-mortem signs of heartwater are hydropericardium, hydrothorax (Figure 5) and oedema of the lungs and brain; however, some or all of these signs may be absent and a final diagnosis depends on the observation of *E. ruminantium* colonies in the cytoplasm of brain endothelial cells. The normal procedure is to examine brain smears after staining with Diff-Quik (a commercial Giemsa-type stain), but veterinarians faced with sick animals which may have heartwater normally treat them with tetracycline, which makes colonies of the organism more difficult to detect at any subsequent post-mortem. [23][32]

E. *ruminantium* occurs as clumps of reddish-purple to blue, coccoid to pleomorphic organisms inside capillary endothelial cells. These organisms are often found close to the nucleus, and may be in a ring or horseshow. *E. ruminantium* can also be detected in formalin-fixed brain sections using immunoperoxidase techniques. Only a few colonies may be found in animals with peracute disease.

The colonies are still visible 2 days after death in a brain that has been stored at room temperature (20–25°C) and up to 34 days in a brain that has been stored in a refrigerator at 4°C. [33]

In situation where colonies in brains smears are difficult to be detected, the preferred method is to stain formalin fixed tissue sections with an immunoperoxidase-labelled polyclonal antibody against E. ruminantium, followed by counterstaining with haematoxylin, which enables the infecting organisms to be easily identified within cells from selected tissues, organs and lesions.

Laboratory diagnosis

There are only two types of practical test for diagnosing heartwater in live ruminants: serological tests and molecular genetic tests, and the latter can also be used to diagnose the disease in vector ticks.

Current serological tests are based on the detection of antibodies to the immunodominant *E. ruminantium* outer membrane protein MAPI, and the most reliable of these tests uses a recombinant fragment of MAPI (MAPIB) in an indirect enzyme-linked immunosorbent assay (ELISA) format [33]

Other serological tests available include indirect fluorescent antibody tests, ELISAs and Western blot. However, when the whole *E. ruminantium* is used as antigen, cross-reactions with *Ehrlichia spp*. occur in all of these tests. Serology has limited diagnostic applications

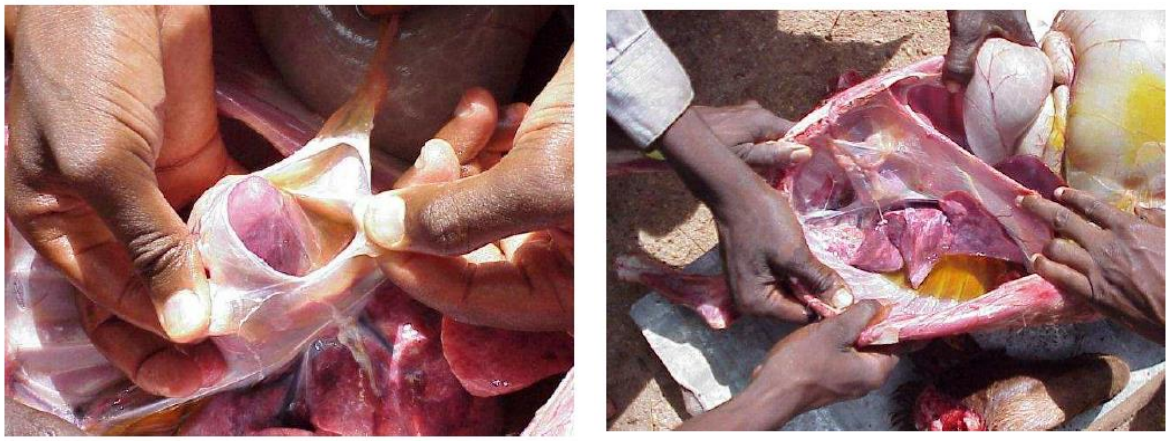

Figure 5: Hydropericardum and Hydrothorax in Heartwater [1]

Molecular tests

The first *E*. *ruminantium*-specific genetic target used for a diagnostic test was a plasmid clone, designated pCS20, from a genomic library of the virulent Crystal Springs isolate from Zimbabwe [3]. The target region consists of two overlapping genes. The tests that have been developed to detect this region all use a variation of PCR amplification, whether directly, in a nested format, in a quantitative real-time format, or in a loop-mediated isothermal amplification (LAMP) format. There are sequence polymorphisms, mostly single nucleotide polymorphisms, among the pCS20 regions of different E. *ruminantium* isolates, but there are also more divergent homologs in all known *Ehrlichia spp*. The pCS20 test can therefore give positive signals with DNA from organisms other than *E*. *ruminantium,* most notably with *E*. *chaffeensis* and *E*. *canis*; nevertheless, when the test is properly calibrated, these signals are an order of magnitude lower than those given by an equivalent concentration of *E*. *ruminantium* DNA. Extensive use of the pCS20 test over more than 20 years has shown that

it can specifically identify *E*. *ruminantium* in domestic animals, wild game and ticks. The quantitative real-time format for this test appears to be the most sensitive for examining field samples, while the LAMP format may be more convenient for less well-equipped laboratories [5][33].

Incidence and Prevalence in Selected Countries

Global

While heartwater has been shown to be endemic in several African countries, there are very limited data on its incidence and prevalence due to several reasons, including the difficulties in diagnosing the disease, the lack of diagnostic capacity in most countries, ongoing use of acaricides and antibiotics in certain regions, the situation of endemic stability in many affected areas which tends to show low incidence of the disease while preventing the introduction of new naïve animals.

Incidence data provided to the OIE or AU-IBAR are generally from the same countries, while the disease is known to occur in much more other countries. However, it can be noted that heartwater has consistently be among the top 11 diseases reported in terms of annual number of outbreaks.

Another observation that could be made from the AU-IBAR report is the fact that countries with better organised veterinary services or countries with commercial farming systems, such as those in Southern Africa. There is also regular report of high number of outbreaks in Somalia, due essentially to the presence of several veterinary charities and international programs that monitor livestock.

Regional

Incidence data by country

Table 5: Number of heartwater outbreaks reported to the OIE between 2005-2015 (Numbers given only for the target countries) Source: OIE.
http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/statusdetail

Country	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
Asia											

Bangladesh	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
India	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Indonesia	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Myanmar (Burma)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Nepal	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Vietnam	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
West Africa											
Burkina Faso	2	1	0	0	0	0	0	0	0	1	0
Ivory Coast	0	0	0	0	0	0	0	0	0	0	0
Mali	0	0	...	...	0	...	...	...	...	...	...
Senegal	1	0	0	0	1	0	0	0	0	1	0
East Africa											
Ethiopia	0	0	0	0	0	1	+	+	0	0	0
Kenya	?	?	?	?	?	?	?	?	?	6	0
Rwanda		...	...	0	0	0	0	0	0	...	...
Tanzania	0	38	114	30	18	23	15	7	11	15	5
Uganda	...	...	...	...	...	...	...	...	...	...	
Southern Africa											
Madagascar	28	4	12	+	+	14	8	6	8	7	0
Malawi	...	+..	+..	...	+..	...	...	0	+..		
Mozambique	5	4	12	5	4	5	7	1	3	5	0
South Africa	250	181	122	231	188	153	145	105	94	55	0

Zambia	…	36	36	89	147	94	0	57	106	97	0

Legend

0	Continuing previous outbreak (s)
...	No information available for this disease
0	Disease absent
?	Disease suspected
+?	Infection/infestation
+..	Disease present but without quantitative data
+	Disease present with quantitative data but with an unknown number of outbreaks
+()	Disease limited to one or more zones
+?()	Infection/infestation limited to one or more zones
?()	Disease suspected but not confirmed and limited to one or more zones

2- AU-IBAR: The number of outbreaks reported to AU-IBAR is included in the Pan African Animal Resources Year Book. (**http://www.au-ibar.org/pan-african-animal-resources-yearbook?showall=&limitstart**=) and can be seen for the countries of interest in Table 6 below.

Table 6: Number of heartwater outbreaks reported to the AU-IBAR from 2005 to 2015 (numbers given only for the target countries). Source: AU-IBAR Year Books.

Country	2005*	2006*	2007*	2008	2009	2010	2011	2012	2013	2014	2015
West Africa											
Burkina Faso										1	
Ivory Coast											
Mali											
Senegal					1						
East Africa											
Ethiopia						1					
Kenya			36	5	51 cases	70 cases	60	45	17	2	
Rwanda											

Tanzania		81	119	237 cases	82 cases	217 cases	10	10	11	14	
Uganda					2						
Southern Africa											
Madagascar		33	36	775 cases	146 cases	175 cases	19				
Malawi											
Mozambique		8	8	4	4	7	8		3	4	
South Africa		174	191	230	70	129		97	101	60	
Zambia			41	11	40	40	74	38	106	97	

*AU-IBAR didn't start yet producing data for Heartwater
**: There were no outbreaks reported, but cases were reported from outbreaks started during the previous year.
NS+ Not specified

Prevalence data by country

Prevalence information of Heartwater (or its vector *A. variegatum*) in the different countries (Data from 2000-2015) is very limited.

Burkina Faso

Year	Area	Species of animal	No. samples tested	% positive	Reference
2008	Békuy Department	Cattle, sheep and goat	2,112 animals 1,612 ticks	An average prevalence by PCR of ticks collected on cattle, sheep and goat of 3.65 (80 animals) 57.9% of ticks were *A. variegatum*	Dr. Adakal PhD (2008)

- Despite the low prevalence, it is clear, from the study conclusion that the disease is a serious problem in Burkina Faso

Ethiopia

Year	Area	Species of animal	No. samples tested	% positive	Reference
2014				*A. variegatum* is reported to account for 40% of the tick population	Kifle et al., 2014
2012	Jimma town	Small scale dairy farming	54	Heartwater was the fourth most important problem identified, after mastitis, internal parasites and lumpy skin disease, mentioned by 5.6% or participants.	Duguma et al., 2012
2012	Abernosa cattle ranch	Ticks	120	Twenty of the ticks (16.6%) were positive for E. ruminantium	Kifle et al., 2014
1996	Abernosa cattle ranch			*A. variegatum* is reported to account for 25% of the tick population.	Kifle et al., 2014

Duguma 2012: **http://idosi.org/gv/GV8(1)12/11.pdf**

Kifle 2014: **http://www.idosi.org/gjms/gjms9(2)14/1.pdf**

Ivory Coast

Year	Area	Species of animal	No. samples tested	% positive	Reference

2002	Central Guinea savannah	N'Dama cattle		Amblyomma: overall prevalence 96%, Seroprevalence of *E. ruminantium:* 31% (95% CI: 26, 36%)	Knopf et al, 2002

Knopf 2002: **http://www.ncbi.nlm.nih.gov/pubmed/11821134**

Kenya

Year	Area	Species of animal	No. samples tested	% positive	Reference
2007-2009	Busia (Western Kenya)	Zebu Cattle	548 recruited, 88 dead	6 dead animals positive for Heartwater (7% of deaths)	de Clare Bronsvoort, 2013 and Thumbi, 2013
2006	Narok District	Sheep and goats	Sheep: 147 Goats: 149	Sheep: 62-85% Goats: 42.5-52%	Wesonga et al, 2006
2001 (15 year retrospective study)	Central Kenya	Sheep	366 (that have died due to disease)	Heartwater was 27% of all parasitic diseases that caused mortality	Kagira et al, 2001
1984-1998	Central Kenya	Cattle	1,413 dead due to disease (177 due to parasites)	TBD: 10.6% of all deaths. Of those, Heartwater was 10.2%	Kanyari et al, 2000

Thumbi 2013: **http://www.ncbi.nlm.nih.gov/pubmed/24010500**

de Clare Bronsvoort 2013: **http://www.ncbi.nlm.nih.gov/pubmed/24000820**

Wesonga 2006: **http://www.ajol.info/index.php/bahpa/article/view/32727**

Kagira 2001: **http://www.ncbi.nlm.nih.gov/pubmed/11811702**

Kanyari 2000: **http://www.ncbi.nlm.nih.gov/pubmed/11131115**

Madagascar

- No recent information was available for Madagascar.

Malawi

Year	Area	Species of animal	No. samples tested	% positive	Reference
1992	Near Lilongwe	Zebu calves	90	Bimodal distribution showing a mixed population of Ab positive and negative animals 77% exposed to *A. variegatum*	Sumption et al, 2003

Sumption 2003: **http://www.ncbi.nlm.nih.gov/pmc/articles/PMC193895/**

Mali

Year	Area	Species of animal	No. samples tested	% positive	Reference
2007	Animals coming from Mali, tested in Senegal	Maure zebus		98%	Mbengue et al, 2007

Mbengue 2007: **http://www.ncbi.nlm.nih.gov/pubmed/17645191**

Mozambique

Year	Area	Species of animal	No. samples tested	% positive	Reference
1997-1998	Northern province of Tete	Goats	332	8.1%	Bekker et al, 2001
1993	Six provinces	Cattle and goats	Cattle: 374 Goats: 388	Cattle: 43% Goats: 30.4%	Asselbergs et al 1993

Bekker 2001: **http://www.ncbi.nlm.nih.gov/pubmed/11427440**

Asselberg 1993: **http://www.ncbi.nlm.nih.gov/pubmed/8236490**

Rwanda

- No recent information was available for Madagascar.

Senegal

- No recent information was available for Madagascar.

South Africa

Year	Area	Species of animal	No. samples tested	% positive	Reference
2011	North West Province	Ticks	42,566	*A. hebraeum* accounted for 17.3% of the ticks. Prevalent mainly in the north eastern region of the province	Spickett et al, 2011
2007-2008	Sweet and sour rangelands in a semi-arid area	Nguni and non-descript cattle	144	All negative	Marufu et al, 2010

Spickett 011: **http://www.ojvr.org/index.php/ojvr/article/viewFile/305/347**

Marufu 2010: **http://www.ncbi.nlm.nih.gov/pubmed/19733490**

Tanzania

Year	Area	Species of animal	No. samples tested	% positive	Reference
2009	North Eastern Tanzania	Sheep and goats	Sheep: 497 Goats: 555	Sheep: 68.6% Goats: 64.7%	Swai et al, 2009a
2008	Manyara Ranch, Monduli District	Cattle	360	50.3	Swai et al, 2008

				2,600 ticks were collected from the cattle. *A. variegatum* accounted for 59.9% of them.	
1999-2001	Tanga region	Cattle	549	56% deaths due to TBD. No mortality due to Heartwater	Swai et al, 2009b

Swai 2009a: **http://www.ncbi.nlm.nih.gov/pubmed/19067218**

Swai 2008: **http://jsava.co.za/index.php/jsava/article/viewFile/247/225**

Swai 2009b: **http://www.ncbi.nlm.nih.gov/pubmed/19091478**

Uganda

Year	Area	Species of animal	No. samples tested	% positive	Reference
2013	Moroto and Kotido districts of Karamoja	Transhumant zebu cattle	24 discussion groups	Heartwater in Moroto was considered one of the most important diseases (after ECF, anaplasmosis and CBPP). Paper includes perceptions but not testing data	Byaruhanga et al

Byaruhanga 2015: **http://www.ncbi.nlm.nih.gov/pubmed/26527312**

Zambia

Year	Area	Species of animal	No. samples tested	% positive	Reference
2010-2011	Mungwi	Cattle	299	Only 1 sample positive, but *A. variegatum* were identified from 52.9% of the sampled	Tembo, 2012

				animals (sensitivity of the test used is questioned)	
2007 - 2008	Three provinces (Eastern, Lusaka and Central)	Cattle	Eastern Province: 422 Central Province: 151 Lusaka Province: 65	Prevalence dry season / wet season: Eastern province: 5.45 – 18.96 Central province: 37.75- 33.93 Lusaka province: 45.31-29.27 Overal: 17.11-23.78	Simuunza, 2009
2004	Six districts in communal grazing semi-arid areas: Gwembe, Lumsemfwa, Luangwa and sourthern parts of the Western province	Goats	451	40.1% *A. variegatum* constituted 42.4% of the ticks	Ahmadu et al, 2004

Tembo 2012: **http://repository.up.ac.za/bitstream/handle/2263/26218/dissertation.pdf?sequence=1**

Simuunza 2009: **http://theses.gla.ac.uk/1240/1/2009simuunzaphd.pdf**

Ahmadu 2004: **http://www.ojvr.org/index.php/ojvr/article/viewFile/279/258**

Economic and Social Impacts at Global and Regional Levels, and in Selected Countries

Since heartwater is so common in the endemic areas of Africa, farmers are usually unwilling or unable to pay for definitive diagnoses, so it is difficult to quantify the economic impact of the disease [4]. An estimate in the literature applied to the Southern African Development Community, gives an indication of total animal production losses from the disease to be averaging US$48 million annually.

The extensive study and report by Minjauw et al. [20] provide useful information on the impact of Heartwater on poor livestock keepers in Africa. See Table 7.

Table 7: Estimated annual costs of tick-borne diseases in US$ [20]

Country	Cost
Angola	800 000
Botswana	400 000
Malawi	400 000
Mozambique	3 000 000
South Africa	31 600 000
Swaziland	1 900 000
Tanzania	2 900 000
Zambia	3 700 000

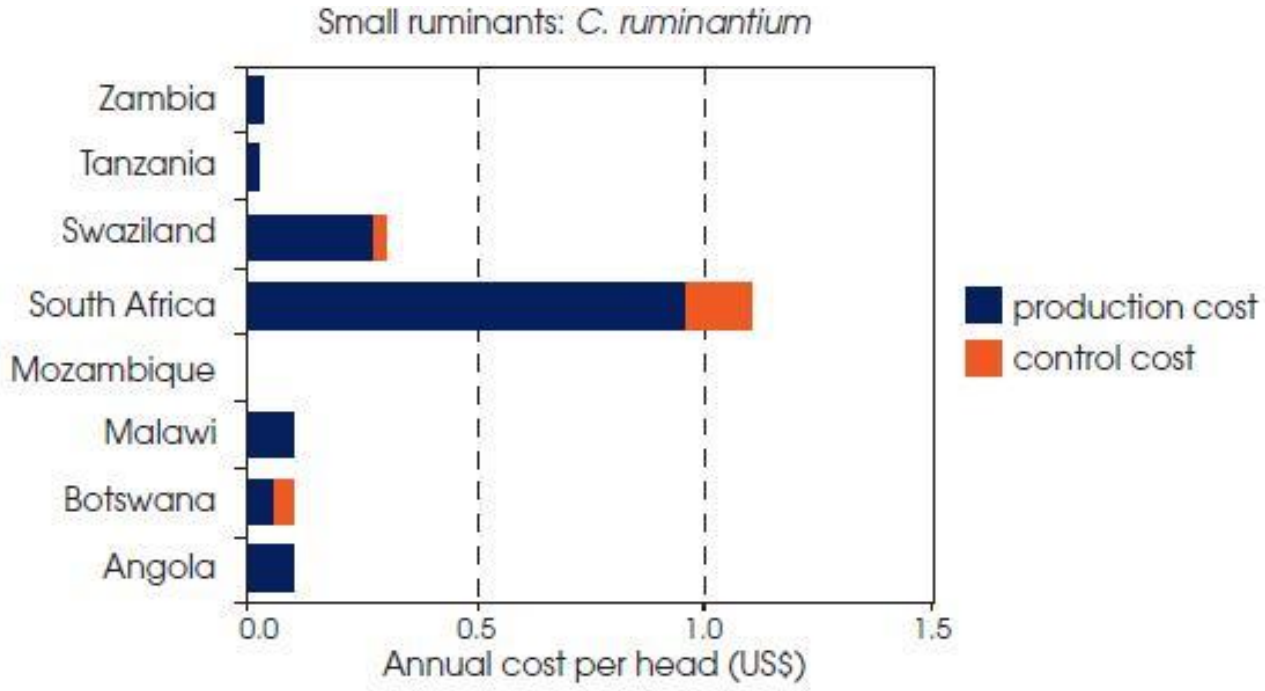

Figure 6: Annual costs per head associated with Heartwater in small ruminants [20]

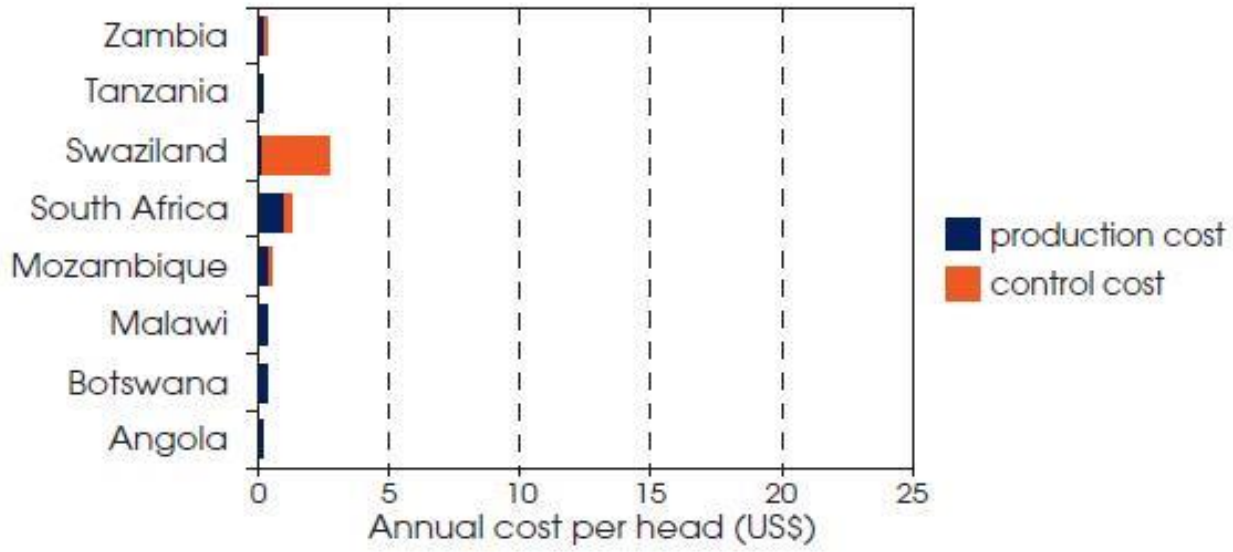

Figure 7: Annual costs per head associated with Heartwater in cattle [20]

Except in the Republic of South Africa, E. *ruminantium* has a relatively small national impact, but since it is the only TBD to cause mortality in small ruminants, it can be of particular concern to commercial producers of these animals. The per head costs for *E*. *ruminantium* are generally higher for cattle than for small ruminants, suggesting that very little preventive action is taken for indigenous small ruminants. The reasons for this include the fact that turnover in small ruminant systems is relatively high, and, because reproduction rates are high compared to those of cattle, small ruminant flocks recover faster from mortality caused by disease outbreaks.

Kivaria [16] established that the annual economic losses due to heartwater in Tanzania were totaling USD22, 43 million, of which control through chemotherapy and acaricides counted for USD7 million, mortality USD 8.8 million and production losses (milk and live weight more than USD6 million [16]

Disease Prevention and Control Methods

In free countries, it is important to prevent entry of infected animals or vectors. *E. ruminantium* cannot survive outside a living host for more than a few hours at room temperature. For this reason, heartwater is usually introduced in infected animals, including asymptomatic carriers, or in ticks. In heartwater-free countries, susceptible ruminants from endemic regions are tested before importation. All animals that may carry Amblyomma, including non-ruminant species, must be inspected for ticks before entry. In addition, ticks may be carried into a country on illegally imported animals or migrating birds.

In endemic areas, treatment, tick control and immunisation are used for the control of Heartwater.

Treatment (Control)

In endemic areas, animals with heartwater can be treated with antibiotics. Tetracycline is effective during the early, febrile stages of this disease, but animals often die before treatment can be administered. Antibiotic treatment alone is not always successful in later stages.

Various formulations of the tetracyclines are almost invariably used for treatment of heartwater, administered at the rate of 8-10 mg/kg [28].

Treatment of small stock showing clinical signs has resulted in a 48% recovery rate (Du Plessis et at. 1994). Uleron, the first agent found to be effective in treating heartwater in sheep, is one of many sulphonamides used with good results. However, repeated doses may be necessary, and it is believed that tetracyclines are more effective [28].

Animals moved into endemic areas may be protected by prophylactic treatment with tetracycline.

Prophylaxis (Prevention)

Tick control

In endemic regions, heartwater can be prevented by tick control and vaccination.

Tick control can be either intensive or strategic.

Intensive tick control has largely fallen into disuse. The objective was to control all stages of ticks throughout the year and it was a system advocated for marginal areas where *Amblyomma spp*. were only found occasionally. The logistics and expense of such operations are formidable if they are applied over large areas, and acaricide resistance is a widespread problem. The main disadvantage, however, is that animals may lose all immunity to tick-borne diseases because of the lack of a natural challenge. Any breakdown of the intensive control regimen then results in heavy losses from heartwater and other tick borne diseases.

Strategic tick control implies the control of tick numbers so that natural infection of livestock occurs and high levels of immunity are maintained. The aim is to achieve an epidemiologically stable situation with respect to heartwater by the regulation of the numbers of ticks present so as to prevent the debilitating effects of severe tick infestations. It is usually recommended that animals should be dipped only if they are carrying, on average, more than 10 adult Amblyomma ticks each. The animals should be monitored weekly in summer and autumn immediately after or during periods of good rains, and every two to three weeks in winter. It has been shown that this approach can lead to endemic (or enzootic) stability, even when the strategy is somewhat erratically applied. Economic studies have demonstrated that strategic tick control is both a more economical and a more practical option for limiting losses from heartwater and other tick-borne diseases. In fact, the counter-intuitive observation has been made that direct tick-borne disease losses increase with increasing use of acaricides. [3][8]

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 [7]

A point worth noting is that heartwater can be eradicated from a region by eliminating its vectors. *Amblyomma* ticks can be difficult to eradicate due to their high rate of reproduction, the wide variety of hosts they infest, and acaricide resistance. A regional program (The Caribbean Amblyomma Program) has been established to eradicate *Amblyomma variegatum* ticks from English and Dutch-speaking islands in the Caribbean. A complementary eradication program (POSEIDOM Vétérinaire Programme) has been conducted on French-speaking islands. To date, these programs have succeeded in reducing the numbers of ticks on some islands and eradicating them from others, but complete eradication throughout the Caribbean remains elusive. [24]

Immunisation

Although vaccination methods described to date include infection and treatment, attenuated, DNA and inactivated vaccines, the only commercially available method, used in South Africa since decades, is the infection-and-treatment method which consists in inoculating intravenously virulent Ball 3 strain of ER (blood of an infected animal or tick homogenate) and subsequently treating with tetracycline.

Disease situation and government policies by country

Tables 8 and 9 below have been completed with the information received so far from the questionnaires sent to the DG and DVS.

Table 8 covers the disease situation (if it is notifiable or not), the presence of official surveillance and/or control programs, and the treatment situation. Table 9 table refers to vaccination.

The definitions that were given to the respondents are:

[1]Surveillance: is the systematic ongoing collection, collation and analysis of data and the timely dissemination of information to those who need to know so that action can be taken.

[2]Control: a programme which is approved, and managed or supervised by the Veterinary Authority of a country for the purpose of controlling a vector, pathogen or disease by specific measures applied throughout that country, or within a zone or compartment of that country.

Table 8: Feasibility of OIE recommended sanitary prevention measures in smallholder poultry settings

Country	Notifiable (yes/no)	Official surveillance[1] program (yes/no) If yes, active or passive	Official control[2] program (yes/no)	Treatment (Chemotherapy)	
				Treatment authorised (yes/no)	Frequently practiced (yes/no)
Burkina Faso					
Côte d'Ivoire (Ivory Coast)	Yes	-	No	-	-
Ethiopia					

Kenya	Yes	Yes, passive	No	Yes	Yes
Madagascar					
Malawi	No	No	Yes	Yes	Yes
Mali	-	-	-	-	-
Tanzania	No	Yes, passive	Yes	Yes	Yes
Uganda	No	No	No	N/A	N/A
Zambia	Yes	Yes, passive	YEs	Yes	Yes

No information from Mozambique, Rwanda, Senegal and South Africa
- Questionnaire received, but no information provided

Table 9: Feasibility of OIE recommended sanitary prevention measures in smallholder poultry settings

Country	Vaccination			
	Compulsory vaccination (yes/no)	Who pays for the vaccine (Government, farmers, combination, others-specify)	Who delivers the vaccine (official, private vaccinators or both)	Species vaccinated (cattle, sheep, goats, pigs, poultry)
Burkina Faso				
Côte d'Ivoire (Ivory Coast)	No	-	-	-
Ethiopia				
Kenya	N/A	N/A	N/A	N/A
Madagascar				
Malawi	No	N/A	N/A	N/A
Mali	-	-	-	-

Tanzania	No	Not done	Not done	N/A
Uganda	No	Never vaccinated	N/A	N/A
Zambia	No	N/A	N/A	N/A

No information from Mozambique, Rwanda, Senegal and South Africa
- Questionnaire received, but no information provided

Vaccines Available

The only commercially available procedure for heartwater immunisation is a more than 50-year old infection and treatment technique developed in South Africa.

The only 'vaccine' currently commercially available is a cryopreserved preparation of blood from a sheep infected with virulent *E. ruminantium* organisms of the Ball-3 isolate [33]. The blood is injected intravenously in animals to be immunized, the rectal temperature is monitored daily, and antibiotic treatment is administered at the proper time. The infective blood must be preserved on dry ice or in liquid nitrogen and thawed shortly before inoculation, and the whole procedure must be supervised by trained staff. [21].

The Ball 3 stock was originally isolated in the Limpopo Province of South Africa and was chosen as the vaccine stock because it produces an early temperature rise several days before any other serious clinical signs appear. This makes it relatively easy to decide when to treat. [21]

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 [12].

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. Where daily temperatures cannot feasibly be monitored, the antibiotic may be administered by the block method [12]. Here, small ruminants are treated, without reference to febrile reactions, on the 11th (sheep) or 12th (goats) day after vaccination. However, experience in South Africa has shown that Angora goats should not be treated by the block method but, like other valuable animals, should be monitored for rise in temperature, then treated [3].

The duration of immunity is uncertain, and because live organisms are involved the procedure cannot be used in non-endemic areas. The procedure is, however, successfully used to protect susceptible animals against the disease, especially when they are first introduced into endemic areas, or if they are particularly valuable.

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 [13]. An improved vaccine is clearly needed.

Commercial vaccines manufactured in Africa

Table 10: Heartwater vaccines manufactured in Africa

Manufacturer	Country	Name & Strain	Vaccine Type	Countries distribution
Onderstepoort Biological product	South Africa	The Ball 3 stock	Blood vaccine, infection and treatment	South Africa, with sporadic sales in neighbouring countries

Commercial vaccines imported into Africa

The information summarised in Table 11, is based on a questionnaire send to the Director of Veterinary Services office and regulators of the countries of interest. Note that some vaccines might have been imported under DVS dispensation, and they are not necessary licensed in the country.

To the best of our knowledge, none of the target countries, in the exception of South Africa, Zambia and to a limited extend Mozambique, practices vaccination.

None of the countries reported to have imported the vaccine, and this has been confirmed by the questionnaires.

Table 11: Vaccine imported into the different countries

Country	Vaccine name	Strain or type	Country of origin	Doses imported 2015	Doses imported 2014	Doses imported 2013	Doses imported 2012
Côte d'Ivoire (Ivory Coast)	-	-	-	-	-	-	-

Kenya	-	-	-	-	-	-	-
Malawi	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Mali	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Rwanda	-	-	-	-	-	-	-
Tanzania	-	-	-	-	-	-	-
Uganda	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Zambia	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Characteristics of Ideal Vaccine Candidates for Smallholders

Table 12 below provides the target minimum attributes of a heartwater vaccine, as well as those of an ideal vaccine

Table 12: Target Product Profile (TPP) heartwater – Proposal:

	Attribute	Minimum (current available vaccine)	Ideal
1	Antigen	Immunogen with protective antigens of E. ruminantium or A. centrale that protects against E. ruminantium infection	Immunogen capable of providing full protection in cattle against E. ruminantium infection
2	Indication for use	For active immunization of cattle & water buffaloes	For active immunization of cattle, water buffalos and all susceptible animals
3	Recommended species	Cattle, Water buffaloes	All E. ruminantium susceptible livestock
4	Recommended dose	2 ml	1 ml
5	Pharmaceutical form	Reconstituted injectable solution/suspension (freeze-dried vaccine) or ready to use solution (inactivated vaccine)	Ready to use solution/suspension
6	Route of administration	intramuscular	SC, Intramuscular or pour on

7	Regimen - primary vaccination	Single dose	Single lifetime dose
8	Regimen - booster	Single annual booster	Lifelong immunity after primary vaccination
9	Epidemiological relevance	Protection against all geographically distinct strains of *E. ruminantium*	Protection against RVF and prevention of virus transmission
10	Recommended age at first vaccination	• Animals over 3 months: one injection	From 1-2 months of age
11	Onset of immunity	2-3 weeks following primary vaccination	One week following primary vaccination
12	Duration of immunity	At least 1 year	Lifelong immunity
13	Expected efficacy	To prevent disease & prevent mortality.	To prevent infection and transmission. No disease & no mortality in vaccinated animals after virulent challenge.
14	Expected safety	In animals under 6 months of age, a transient pyrexia reaction can occur. A transient nodular reaction of varying importance, may appear at the injection site, it progressively disappears within 1 to 2 months. Only vaccinate pregnant animals on emergency.	No post-vaccinal reactions at any age. Safe for pregnant animals. No carrier form in vaccinated animals
15	Withdrawal period	Nil	Nil
16	Special requirements for animals	Do not vaccinate un-healthy animals	Do not vaccinate un-healthy animals DIVA
17	Special requirements for persons	None	None

18	Package size	50 doses	Multiple pack size from 10 doses
19	Price to end user	Not more than $0.50/dose	$0.20/dose at end user
20	Storage condition and shelf-life as packaged for sale	12 months at 4-8° C	24 months 4-8° C and/or 48 hours at 30° C
21	In-use stability	1 hour	24 hours

Overall conclusion for improved Heartwater control through vaccination

South Africa continues to be the only country with a form of vaccination for a disease that is widespread throughout the whole Sub-Saharan Africa, and in some accounts considered to be the second most economically important TBD after ECF. Given the known limitation of treatment and vector control through the use of acaricides, a vaccine has been considered for many years as the most effective control strategy.

From the present report it is clear that none of the vaccines developed to date has been satisfactory in ensuring commercial production. There are however a number of elements that could be considered for supporting the development of an effective vaccine:

- There is a need to validate molecular tools for rapid strain matching, so that vaccine compatibility and suitability cold be ensured
- Evaluate inactivated form of vaccines based on broad spectrum isolates or stock, such as Welgevonden, using knowledge accumulated by the different groups
- Evaluate combination vaccination program, with priming with inactivated vaccine and boosting with live attenuated vaccine
- Conduct a full development on the attenuated Welgevonden vaccine, essentially assess reversion to virulence and possibly lyophilisation
- Development of robust challenge models, which should preferably include a tick challenge

- Reverse vaccinology for the identification and use of antigens that are targets of cellular immune responses through the work of some groups, such as University of Florida, could be an avenue for new vaccine development

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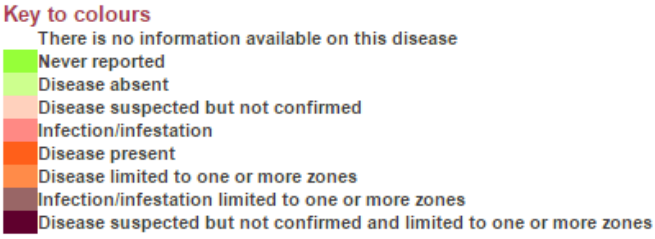
ANNEX 1: Additional data on disease presence and incidence

Reports to OIE on heartwater:



When different animal health statuses between domestic and wild animal population are provided, the box is split in two: the upper part for domestic animals, and the lower part for wild animals.

Heartwater in Western Africa: Burkina Faso, Ivory Coast, Mali and Senegal

Heartwater in Eastern Africa: Ethiopia, Kenya, Rwanda, Tanzania and Uganda

Heartwater in Southern Africa: Madagascar, Malawi, Mozambique, South Africa and Zambia