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Heartwater – *Ehrlichia ruminantium* infection

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Summary
Heartwater is a notifiable disease that is listed by the World Organisation for Animal Health. It is caused by *Ehrlichia ruminantium*, an obligately intracellular Gram-negative bacterium in the order Rickettsiales and the family Anaplasmataceae. The disease is borne by ticks in the genus *Amblyomma* and causes heartwater, or cowdriosis, in wild and domestic ruminants, primarily in Africa, but also in parts of the Caribbean. The disease was recognised in South Africa in the 19th Century and determined to be tick borne in 1900, while the organism was identified in 1925 and first cultured *in vitro* in 1985. This latter achievement boosted research into the disease at a time when biology was moving into the molecular genetic age. Over the last 20 years, there have been significant improvements in our understanding of *E. ruminantium*, yielding major advances in diagnosis, epidemiology, genetic characterisation, phylogeny, immunology, and vaccine development. The organism is genetically highly variable; this has important implications for future control measures, and is making it difficult to develop an effective vaccine for protection against tick challenge. Research is continuing into three different types of vaccine, inactivated, attenuated, and recombinant, and the current state of development of each is discussed.

Keywords
Amblyomma hebraeum – *Amblyomma variegatum* – Attenuated vaccine – Control – Cowdriosis – Diagnosis – Economic importance – *Ehrlichia ruminantium* – Heartwater – Inactivated vaccine – Occurrence – Recombinant vaccine – Tick-borne disease.

Aetiology

Heartwater is listed by the World Organisation of Animal Health as a notifiable disease (1). It was known in South Africa for nearly 90 years before the causative organism was identified in 1925 as a rickettsia, originally named *Rickettsia ruminantium* (2, 3). The name was later changed to *Cowdria ruminantium* (4), from which arose the term 'cowdriosis'. Molecular phylogenetic studies of the Rickettsiales in the 1990s uncovered the real evolutionary relationships within the order and the organism was reclassified as *Ehrlichia ruminantium* in the family Anaplasmataceae (5). *Ehrlichia ruminantium*, which is transmitted by *Amblyomma* ticks, is obligately intracellular, infects cattle, sheep, goats and some wild ruminants, and is frequently fatal. A comprehensive account of the history and biology of *E. ruminantium* can be found elsewhere (6).

Economic and social importance

Heartwater is a serious economic problem wherever it occurs, in an enormous area covering most of sub-Saharan Africa, its offshore islands, and several islands in the Caribbean. The disease generally prevents livestock farmers from upgrading their herds to modern high-yielding breeds, as these are more susceptible to infection than traditional stock breeds, which often have a measure of resistance (7). 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. The only estimates in the literature apply to the Southern African Development Community, where total animal production losses from the disease are thought to average US$48 million annually (8, 9).

Much of the endemic heartwater area in Africa tends to be dry, which seriously restricts crop production, while animal production is not so badly affected. Data from South Africa illustrate the problem; in 2011, a typical year, agricultural production used about 50% of the country's available water, with the remainder going to industrial and household use (10). The total economic value derived from agriculture was almost evenly divided between crop growing and animal production (11), but far more than half of the agricultural water was devoted to growing crops. Animal production largely uses naturally watered rangeland, which is unsuitable for crop production because of its low rainfall and poor quality soils (12). In areas subject to these climatic and environmental constraints, an adequate human diet relies heavily on animal production, and the situation is unlikely to change in the foreseeable future, except perhaps to get more extreme. Heartwater is therefore a constant threat to food security in many parts of Africa.

Clinical signs

The incubation period of heartwater averages 18 days in cattle and 14 days in sheep and goats; the clinical signs are diverse, and the disease severity varies from peracute to clinically inapparent. The breed and age of the infected animal affect the course of the infection. Angora and Boer goats, for example, frequently exhibit the peracute form, and infected animals may collapse suddenly and die in convulsions without showing any other signs (13). Two other important factors are the severity of the tick challenge and the virulence of the genotype of the *E. ruminantium* strain involved. The variety of signs and effects makes the clinical diagnosis of heartwater in live animals difficult, especially as many of the clinical signs are not definitively diagnostic (14). The normal signs are, in order of increasing severity: elevated temperature, loss of appetite, heavy breathing, hanging head, stiff gait, depression, exaggerated blinking and chewing movements, anorexia, hyperaesthesia, lacrimation, convulsions, recumbency and death. Most of these signs would not individually constitute a definitive diagnosis, which ultimately depends upon identification of *E. ruminantium* at the post-mortem examination and/or after laboratory diagnosis. A more complete treatment of the topic can be found elsewhere (15).

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 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. In this situation the preferred method is to stain formalin-fixed tissue sections with an immunoperoxidase-labelled polyclonal antibody against *E. ruminantium*, followed by counterstaining with haematoxylin (16), which enables the infecting organisms to be easily identified within cells from selected tissues, organs and lesions. In 2012, staff of the Pathology Department at the Faculty of Veterinary Science in Pretoria, South Africa, confirmed 43 cases of heartwater at post-mortem: 20 by normal brain smear staining and 23 by antibody staining (S. Clift, personal communication). More extensive details of the pathology of the disease are found elsewhere (15).

Ante-mortem 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.

The first serological test for heartwater used peritoneal macrophages from *E. ruminantium*-infected mice to detect antibodies in sera from infected animals (17), but it was quickly observed that false-positive reactions were common (18). Current serological tests are based on the detection of antibodies to the immunodominant *E. ruminantium* outer membrane protein MAP1, and the most reliable of these tests uses a recombinant fragment of MAP1 (MAP1B) in an indirect enzyme-linked immunosorbent assay (ELISA) format (19). It must be emphasised, however, that all serological tests for *E. ruminantium* may exhibit false-positive reactions, owing to the presence of closely related homologs of *map*1 in other Anaplasmataceae species (20, 21, 22). Serological tests for heartwater also exhibit false-negative results, mostly in cattle, as antibody levels are often too low to be detected, even in animals that are under continuous natural challenge by infected ticks (23, 24). It has been demonstrated that results from the MAP1B ELISA test do not correlate well with those from a nested pCS20 polymerase chain reaction (PCR) test (25), and it is this latter test that the authors will discuss next.

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 (26). The target region consists of two overlapping genes (27). The tests that have been developed to detect this region all use a variation of PCR amplification, whether directly (27), in a nested format (28), in a quantitative real-time format (29), or in a loop-mediated isothermal amplification (LAMP) format (30). 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.

Epidemiology in Africa

Heartwater occurs in wild and domestic ruminants wherever the tick vectors are present, which is primarily in Africa. The natural reservoir consists of numerous species of African wild ruminants (6), but the disease can also be maintained solely within a domestic stock population (31). The vectors are all ticks in the genus *Amblyomma* and the distribution of the disease in Africa coincides with that of the two most important vector species, *A. variegatum* and *A. hebraeum* (32). The endemic area covers most of sub-Saharan Africa (except for the very dry south-west) and the islands of Madagascar, Mauritius, Reunion, Grande Comore and São Tomé (33, 34).

Epidemiology in the New World

Amblyomma variegatum was inadvertently introduced into the Caribbean, probably on heartwater-infected cattle in the 18th Century (35). While the disease has only become established on the islands of Guadeloupe, Antigua and Marie-Galante (36, 37), the tick vector has spread to 14 islands as a result of inter-island trade in tick-infested livestock and the transport of larvae and nymphs by the African cattle egret (*Bubulcus ibis*) (38). Cattle egrets migrate as far as the American mainland so both *A. variegatum* and *E. ruminantium* could continue to spread. The Caribbean *Amblyomma* Programme (CAP), launched in 1994, aimed to reduce this risk by eradicating *A. variegatum* from the eastern Caribbean. Nine islands participated in the programme and when it ended in 2008 four islands had been cleared of *A. variegatum* (39). Guadeloupe, Antigua and Marie-Galante were not included in the CAP strategy and still represent a reservoir for both *A. variegatum* and *E. ruminantium* (36). The risk that endemic heartwater could become established in the Americas is very real: the climate of much of tropical and subtropical North, Central and South America is suitable for *A. variegatum*; the native American *A. maculatum* is capable of acting as an effective experimental vector for *E. ruminantium* (40); and native American white-tailed deer (*Odocoileus virginianus*) are known to be susceptible to *E. ruminantium* infection and could therefore act as a natural reservoir (41).

Vector biology

All the vectors of heartwater are three-host ticks which become infected within two to four days of feeding on infected hosts (42, 43). Transstadial transmission of *E. ruminantium* takes place between nymphal and adult ticks (44) but transovarial transmission does not occur in the field. Infected nymphs and larvae only become infective in the following instar (32), and infective nymphs or adults transmit *E. ruminantium* to susceptible hosts without losing the infection. Male *A. hebraeum* also transfer the infection when moving from infected to susceptible animals in search of mates (44).

Vector competence

The intensity of the field challenge in an endemic area depends principally on the population densities of vector ticks and their efficiencies as vectors. Since population densities are heavily influenced by temperature and humidity (45), this leads, in most of Africa, to a peak in *E. ruminantium* infections after good rains. Infection rates in vector tick populations in endemic areas seem to be very variable, with figures from 11.2% to 40.9% being reported (46, 47, 48, 49). It is known, however, that larvae and nymphs fed experimentally on *E. ruminantium*-infected sheep develop infection rates of 100% in the following instar (47), which suggests that, in the field, many ticks feed during the larval or nymphal stages on non-susceptible or non-infected hosts. In a highly managed farm environment, apparently healthy stock may constitute a dangerous disease reservoir; animals treated for heartwater can carry *E. ruminantium* organisms at very low levels and have been shown to remain infective to ticks for about a year (50, 51). These observations show the danger posed by unsuspected carrier animals being moved to heartwater-free areas.

Control

Four methods have been employed for the control of heartwater: tick control, farming with resistant stock, antibiotic treatment, and immunisation.

Tick control

Intensive tick control with acaricides requires highly efficient management to succeed. It was in common use at one time but two unforeseen factors led to the abandonment of this method. First, in the absence of natural challenge, animals in controlled areas rapidly lost their naturally developed immunity to the disease. Any breakdown of the intensive control which allowed an increase in the tick population then led to serious losses from heartwater and other tick-borne diseases (52). Secondly, and more importantly, ticks rapidly developed resistance to acaricides.

Strategic tick control, which involves dipping to control the numbers of ticks on the livestock so that their natural immunity is maintained, is still in use, and the technique can lead to endemic stability. Stability can easily be lost, however, as a result of common events. Seasonal increases in rainfall can augment the tick population; new livestock with different immune capabilities may be introduced (53); and new strains of *E. ruminantium* may be introduced, against which immunity to the existent prevalent strains offers no or limited protection. Strategic tick control is impractical to maintain if the livestock range over a large area, and then losses will inevitably occur if the species is highly susceptible to the disease. This situation applies, for example, to the Angora goat industry in the Eastern Cape province of South Africa.

In summary, it seems unlikely that chemical tick control can ever be an effective method for controlling heartwater, or indeed any tick-borne disease, mainly because acaricide resistance will inevitably develop (54). An alternative method of tick control, which has been under investigation for more than 20 years, is the use of a recombinant anti-tick vaccine. The research has yet to produce a commercially viable product, but this could become the approach of choice in the future (55).

Use of resistant stock

Some breeds of livestock in heartwater-endemic areas appear to be relatively resistant to *E. ruminantium* as the result of long-term selection. For example, *Bos taurus* cattle are less resistant than *B. indicus* (Zebu) breeds and, although the latter still become infected, the disease outcome is often less severe than in the former (31). Unfortunately, the more resistant traditional breeds are generally less productive than modern, higher-yielding but less-resistant breeds (7). There have been attempts to breed goats for resistance in the Caribbean (56) but there are no recent reports of the outcome of this programme.

Antibiotic treatment

Acute *E. ruminantium* infection is normally treated with oxytetracycline, and during the early 1950s the recommended dose was 2.5 mg/kg live weight, repeated after 24 h. More recently, it has been recommended that two treatments with 20 mg/kg be given on successive days; despite this, it is said that the bacterium is not actually resistant to the drug (15). Long-acting oxytetracycline is widely used as a prophylactic treatment when new stock are introduced into an endemic area, to act as protection while allowing the stock to develop some natural immunity (57). Commercial Angora goat farmers in the Eastern Cape province of South Africa routinely use prophylaxis with short-acting oxytetracyclines, given fortnightly at 3 mg/kg, because the animals are highly susceptible to heartwater. This is a very expensive option, because of the cost of the drug itself and the logistical cost of rounding the animals up every fortnight for treatment. It seems inevitable that antibiotic resistance will eventually develop, which will make it impossible to farm with Angora goats in the heartwater-endemic area unless an effective vaccine becomes available.

Immunisation

The only viable long-term method for controlling heartwater is to stimulate protective immunity by the development of an effective vaccine. Immunisation is, in fact, the most widely used control method in southern Africa and, although the treatment is sold as a vaccine, it is in reality a cryopreserved preparation of sheep blood (58) containing virulent infective *E. ruminantium* organisms of the Ball 3 genotype (59). The vaccine is administered intravenously and the temperatures of the injected animals are monitored daily. Antibiotic treatment is then given when the infection becomes established but before serious disease occurs. There are several problems with this procedure. The blood vaccine must be held at a temperature well below freezing until immediately before use because the bacteria rapidly lose infectivity once thawed (60). This is a serious logistical problem in remote rural areas in the tropics and subtropics. In addition, the whole procedure requires supervision by trained staff to administer the vaccine intravenously and to perform subsequent monitoring. As with all live vaccines, the procedure can only be used in areas where the disease is endemic, and even then there may be political reluctance to allow the importation of new immunotypes of the organism. Finally, and most seriously, the duration and effectiveness of immunity are highly variable. This is because there are numerous immunotypes of *E. ruminantium* circulating in the field, and cross-protection between them ranges from

total to minimal (61). There is an urgent need for a safe, effective and cost-efficient new vaccine against heartwater.

New vaccine development

Three different types of vaccine are under development: attenuated, inactivated and recombinant (62, 63). The first of these types could not be used to prevent the spread of heartwater in a non-endemic area, but the other two types would potentially be usable anywhere. If a future vaccination campaign is to be successful, the vaccine must be effective against the different immunotypes of *E. ruminantium* present in the control area, hence complementary research is in progress to determine the nature and distribution of antigenic variants in the field in Africa and the Caribbean (64, 65).

Inactivated vaccine development

This vaccine uses *E. ruminantium* elementary bodies (dense-cored infectious bacterial cells), partially purified from bovine endothelial cell culture. The bacteria are chemically inactivated, formulated with a suitable adjuvant, and administered subcutaneously. The original vaccine was developed in Guadeloupe, using the local Gardel isolate (66), and a modified version was subsequently produced in Zimbabwe, using the local Crystal Springs isolate (67). An early problem with the inactivated vaccine was the difficulty, and therefore high cost, of preparing enough of the antigenic material for large-scale use. This has largely been solved by the development of a semi-industrial mass production process at a cost of around US$0.14 per dose (63, 68).

The experimental inactivated vaccines tested so far protect 50% to 100% of immunised animals against death after a normally lethal homologous needle challenge, but after a heterologous needle challenge, or a normal tick challenge in the field, protection is far less effective (69, 70). A major reason for this appears to be that there is a great deal of non-cross-protective immunogenic diversity among *E. ruminantium* strains in the wild, and – to make matters worse – the different genotypes mutate and recombine to generate further diversity (71, 72). One way to deal with this is to formulate customised vaccines incorporating the immunotypes from the target region, and this approach has shown some success in Burkina Faso (73). A technical problem afflicting all heartwater vaccine development is that a natural tick challenge is much more virulent than an experimental needle challenge (61, 74). This problem requires that a method be developed for delivering quantified experimental challenges with infected ticks.

Attenuated vaccine development

An attenuated vaccine uses an avirulent live pathogen which does not cause clinical disease but which still stimulates immunity against virulent forms of the organism. Three strains of *E. ruminantium* have been attenuated: Senegal, from West Africa; Gardel, from Guadeloupe; and Welgevonden, from South Africa. The attenuated Senegal vaccine (75) completely protects sheep and goats against a homologous needle challenge but in field trials the levels of protection are lower; in two separate studies the vaccine provided reductions in mortality from 70% to 43% (76), and from 100% to 25% (70). There are no records of the attenuated Gardel isolate being used in vaccine trials, but it is known that immunisation (by infection and treatment) against virulent Gardel provides only limited cross-protection against virulent African strains (61).

The attenuated Welgevonden vaccine provides 100% protection to Merino sheep and Boer goats against a lethal needle challenge with the homologous strain or with one of four different heterologous strains (77). There are also preliminary indications that the attenuated organism can be transmitted by ticks to naive sheep which then show immunity. Further experiments in Merino sheep showed that the protection against homologous challenge persisted at 100% for a minimum of six months, falling to 80% after one year, although under normal field conditions, with continuous natural challenge, the 100% immunity level could well have been retained (78). The vaccine was less satisfactory in Angora goats; some of the animals had to be treated after vaccination, and 90% of them were protected against a homologous challenge (78). It must be remembered, however, that this breed is exceptionally sensitive to heartwater. Most recently, the same vaccine was used to immunise Friesian cattle which were subsequently needle-challenged with the Gardel strain; 5/6 cattle (83%) recovered without treatment, while 1/6 required treatment (79). The reason for challenging with Gardel, which is highly virulent in cattle, is that Welgevonden is of low virulence in cattle (62).

Further development of the Welgevonden attenuated vaccine should be a priority. Evidently, it must be tested in the field against a tick challenge and, if it performs well, it should then be formulated and tested for commercial distribution. It would be ideal if this vaccine could be lyophilised, so that a cold chain would not be needed for distribution. However, the retention of infectivity of the organisms after lyophilisation and re-suspension may present difficulties. The fear that the attenuated organisms may revert to virulence is not relevant in the endemic area of southern Africa, where the virulent strain originated and is freely in circulation. There would, however, be reluctance to use this live vaccine in other parts of Africa, or in the Caribbean. To deal with this will require elucidation of the genetic basis of the attenuation and information on the nature and distribution of *E. ruminantium* antigenic variants in a prospective target area.

Recombinant vaccine development

Since inactivated heartwater vaccines can stimulate protective immunity, it is theoretically possible to develop a successful recombinant vaccine if the relevant *E. ruminantium* genes can be identified. A recombinant vaccine should be cheaper to manufacture and easier to store than an inactivated or attenuated vaccine and, most importantly, it could be used to stop an outbreak in a non-endemic area. At least one major problem must be solved before a reliable recombinant vaccine can be produced; protective immunity against *E. ruminantium* involves a T-cell response but there is no reliable known method for identifying the genes responsible for stimulating this response.

Early attempts to develop a recombinant vaccine against *E. ruminantium* used several different genes. The *map*1 gene was one candidate; others were taken from expression libraries after being recognised by *E. ruminantium* antibodies and were selected as candidates if they stimulated proliferation *in vitro* of *E. ruminantium*-immune, peripheral blood mononuclear cells. After cloning in DNA vaccine vectors and testing in mice, the protection levels achieved against homologous needle challenge were unpredictably variable (between 13% and 89%). These early studies are reviewed in detail elsewhere (62).

The variability of the results in mice, and the observation that one experimental DNA vaccine gave much higher protection levels in sheep (80%) than in mice (0%) (80), led to the mouse model being abandoned in favour of using sheep, a natural host. The first experiments, using a cocktail of four genes cloned into a suitable DNA vaccine vector, stimulated 100% protection against a virulent needle challenge with homologous, and five different heterologous, *E. ruminantium* strains (61). Disappointingly, when the vaccine was tested in the field against a natural heartwater-infected tick challenge, levels of protection only reached 20%. In further trials, it was found that each of the four genes provided levels of protection similar to that obtained with the four-gene cocktail, but the protection against field challenge remained poor (81). Heterologous prime-boosting was tried in an attempt to improve the field protection rate. Either recombinant protein obtained by expression of the genes *in vitro*, or recombinant lumpy skin disease virus (rLSDV) incorporating the genes, was administered after the initial vaccination, and a range of immunity parameters were monitored in the experimental animals before challenge. The sheep boosted with recombinant protein showed better *E. ruminantium*-specific lymphocyte proliferation, and higher interferon gamma expression, than those boosted with rLSDV, which was the opposite of what had been expected. Complete protection against an experimental needle challenge was retained in both groups, but protection in the field was not improved (74). Other genes have been tried in a similar DNA vaccine vector, both with and without recombinant protein boosting, but only one provided 100% protection against a homologous needle challenge, and then only when boosted. This gene has not been tested against a field challenge (82).

Identifying those genes that stimulate a protective anti-*E. ruminantium* T-cell response is obviously crucial, and a reverse vaccinology strategy is currently being used to this end. The Welgevonden genome sequence (83) was the starting point, and likely vaccine candidate genes were selected according to their predicted functions. The genes were expressed *in vitro*, and the recombinant proteins tested for their ability to induce interferon gamma production in *E. ruminantium*-immune lymphocytes. The cytokine response profiles of the lymphocytes that responded were monitored. Eleven genes were identified whose protein products induced cytokine responses similar to the recall immune response induced by intact *E. ruminantium* cells (84). Epitope mapping of these products is presently being conducted, with the intention of developing a multi-epitope vaccine (J. Liebenberg, personal communication).

Genotyping

Any future effective heartwater vaccine must provide protection against the different *E. ruminantium* immunotypes circulating in the area where it is to be used, and the traditional method of performing cross-protection studies *in vivo* is logistically far too cumbersome and expensive to be widely applicable. The relatively recent development of different molecular genetic tests for *E. ruminantium* genotypic diversity has found it to be unexpectedly extensive and not geographically localised.

The small-subunit ribosomal RNA (srRNA) gene, widely used for the phylogenetic classification of bacteria, is not sufficiently polymorphic to enable any correlation to be made with pathogenicity (85). Variable-number tandem-repeat loci are unexpectedly common in the *E. ruminantium* genome, and their sizes and repeat numbers have been used for characterisation (64). Their discriminatory capability is, however, less than that available from *map*1 sequence polymorphisms. Multi-locus sequence typing (MLST) using housekeeping genes has shown that most strains display little polymorphism, with evidence of only ancient genomic recombination events; however, a subset of much more variable strains was identified and recombination is presumed to be ongoing (72). This work contributes to a better understanding of evolution and recombination in *E. ruminantium*, which could be crucial for designing control strategies in the future, but MLST is probably not the most convenient technique for genotyping populations prior to vaccination campaigns.

The *map* gene family is the other major characterisation target for *E. ruminantium*; it includes paralogs which are polymorphic, the most variable being *map*1. A comprehensive recent study has been made of *map*1 genes from 80 isolates, plus five other less variable *map* paralogs from a subset of these isolates (65). There is no correlation between the *map*1 genotype and geographical origin, with the same genotype being found on different continents, but there is positive selection pressure for synonymous substitution among the *map*1 genotypes. Although the function of MAP1 is not known, the authors of this study concluded that MAP1 sequences are the best markers currently available to identify different genotypes of *E. ruminantium* across all regions where the organism is found.

Neither the crucial protection-stimulating genes, nor the effective epitopes which they incorporate, have been identified for *E. ruminantium*, and without this information it is impossible to directly link genotypes to cross-protective immunotypes. A possible pragmatic way around this problem could be used when a vaccine that is effective against natural tick challenge becomes available. As many genotypes as possible could be identified in a target area, probably by sampling *E. ruminantium* in the tick population using the *map*1 gene. After introducing vaccinated stock, the *map*1 genotypes of any strains which broke through the vaccine-induced immunity could be identified and modifications made to the vaccine to protect against the local genotypes. Over time, an empirical bank of data would be built up, correlating *E. ruminantium map*1 genotypes with the modified vaccines which induced protection against them.

The crucial question is, how easy would it be to generate new versions of the different types of vaccine to protect against strains with a new *map*1 genotype? The semi-industrial culture process which has been developed for the inactivated vaccine has only been optimised for two stocks, Welgevonden and Gardel, and adapting new isolates to the process could be easy, difficult or impossible, for any of them. For an attenuated vaccine, the same would be true for the process of attenuating a new strain. In the case of a recombinant vaccine, the process should be simpler. It would be possible to identify those sequences (genes or epitopes) in the breakthrough *map*1 genotypes which corresponded to their paralogs in the vaccine and to modify the vaccine accordingly. However, none of the three vaccine types has yet been able to provide effective protection against a virulent tick challenge in the field, so the choice of which one should be adapted to a new area is likely to depend upon which one is the first to be successful in the field in any area.

La cowdriose – Infection par *Ehrlichia ruminantium*

B.A. Allsopp

Résumé

La cowdriose est une maladie à déclaration obligatoire inscrite sur la liste de l'Organisation mondiale de la santé animale. L'agent causal est *Ehrlichia ruminantium*, une bactérie obligatoire intracellulaire à Gram négatif appartenant à l'ordre des Rickettsiales et à la famille des Anaplasmataceae. Cette bactérie transmise par des tiques du genre *Amblyomma* provoque la cowdriose chez les ruminants sauvages et domestiques, principalement en Afrique mais aussi dans certaines régions des Caraïbes. La maladie a été décrite pour la première fois en Afrique du Sud au cours du XIX^{e} siècle ; le rôle de vecteurs des tiques a été élucidé en 1900 ; l'identification du micro-organisme remonte à 1925 mais la première culture *in vitro* a été réalisée en 1985. Ce succès a donné un nouvel élan à la recherche sur cette maladie à une époque où la biologie entrait dans l'ère de la génétique moléculaire. Au cours des 20 dernières années, les connaissances sur *E. ruminantium* ont connu des avancées significatives qui se sont traduites par des améliorations majeures dans les domaines du diagnostic, de l'épidémiologie, de la caractérisation génétique, de la phylogénie, de

l'immunologie et du développement de vaccins. La grande variabilité génétique du micro-organisme a des répercussions importantes sur les mesures de contrôle qui seront prises à l'avenir et entraîne des difficultés particulières pour la mise au point d'un vaccin conférant une protection efficace contre l'exposition aux tiques. La recherche se poursuit, axée sur trois catégories différentes de vaccin : inactivé, vivant atténué et recombinant. L'auteur fait le point sur l'état actuel du développement de ces trois vaccins.

Mots-clés
Amblyomma hebraeum – *Amblyomma variegatum* – Contrôle – Cowdriose – Diagnostic – *Ehrlichia ruminantium* – Impact économique – Incidence – Maladie transmise par les tiques – Vaccin inactivé – Vaccin recombinant – Vaccin vivant atténué.

Cowdriosis – Infección por *Ehrlichia ruminantium*

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Resumen
La cowdriosis es una enfermedad de declaración obligatoria que figura en la lista de la Organización Mundial de Sanidad Animal. Su agente etiológico es *Ehrlichia ruminantium*, bacteria Gram negativa de vida intracelular obligada que pertenece al orden de los Rickettsiales y a la familia Anaplasmataceae. La infección se transmite por garrapatas del género *Amblyomma* y causa cowdriosis en rumiantes salvajes y domésticos, principalmente en África, pero también en algunas zonas del Caribe. La enfermedad fue descrita en Sudáfrica en el siglo XIX, en 1900 se comprobó que la transmitían garrapatas y en 1925 se pudo identificar al microorganismo, que fue cultivado por primera vez *in vitro* en 1985. Aquel logro impulsó las investigaciones sobre la enfermedad, en un momento en que la biología empezaba a entrar en la era de la genética molecular. En los últimos 20 años hemos aprendido mucho acerca de *E. ruminantium*, lo que a su vez ha deparado grandes progresos en los terrenos del diagnóstico, la epidemiología, la caracterización genética, la filogenia, la inmunología y la elaboración de vacunas. El organismo presenta una gran variabilidad genética, cosa que tiene importantes consecuencias de cara a futuras medidas de control y que está dificultando la obtención de una vacuna que proteja eficazmente contra la infección transmitida por la garrapata. Hay investigaciones en curso sobre tres tipos de vacunas: inactivadas, atenuadas y recombinantes, y el autor expone el actual estado de cosas en cada una de esas líneas de trabajo.

Palabras clave
Amblyomma hebraeum – *Amblyomma variegatum* – Aparición de casos – Control – Cowdriosis – Diagnóstico – *Ehrlichia ruminantium* – Enfermedad transmitida por garrapatas – Importancia económica – Vacuna atenuada – Vacuna inactivada – Vacuna recombinante.

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