HEARTWATER - COWDRIOSIS

AETIOLOGY

Classification of the causative agent

Ehrlichia ruminantium (formerly *Cowdria ruminantium*)
Order Rickettsiales, Family Anaplasmataceae

- Small, Gram negative, pleomorphic coccus, and obligate intracellular parasite.
- Strains of *E. ruminantium* are very diverse and vary in virulence: while some strains are highly virulent, others appear to be less-pathogenic.
- *E. ruminantium* has a high level of genomic plasticity. Several different genotypes can co-exist in a geographical area, and may recombine to form new strains.
- *E. ruminantium* multiplies in vascular endothelial cells throughout the body to cause severe vascular compromise.
- It usually occurs in clumps of from less than five to several thousand organisms within the cytoplasm of infected capillary endothelial cells, and can be detected in brain smears by light microscopy.

Resistance to physical and chemical action

Temperature: Heat labile and loses its viability within 12–38 hours at room temperature. Infective stabilities can be cryopreserved in DMSO (dimethyl sulphide) or better yet in sucrose-potassium phosphate-glutamate medium (SPG). Infective half-life of thawed stabilate kept on ice is only 20–30 minutes.

pH: Not applicable.

Disinfectants: Not applicable.

Survival: The heartwater organism is extremely fragile and cannot persist outside of a host for more than a few hours. Because of its fragility, the organism must be stored in dry ice or liquid nitrogen to preserve its infectivity.

EPIDEMIOLOGY

- Heartwater occurs only where its *Amblyomma* tick vectors are present.
- Epidemiology depends on interaction of tick vector, causative agent, and vertebrate hosts.
    - Tick vector: tick infection rates, seasonal changes influencing abundance and activity, and intensity of tick control.
    - Causative Agent: differing genotypes affecting virulence or stimulation of cross-protection.
    - Vertebrate Host: availability of wild animal reservoirs, and age and genetic resistance.
- Because of its extreme fragility, the principal mode of bringing the disease into an area is by introduction of infected ticks or carrier animals. It is not known for how long wild or domestic ruminants can be a source of infection for ticks in nature, but it may be many months. Ticks are a robust reservoir of *E. ruminantium,* and infection can persist in them for at least 15 months. Careful dipping and hand-dressing followed by inspection to ensure the absence of ticks is recommended for animals in transit to heartwater free areas.

Hosts

- All domestic and wild ruminants can be infected, but the former appear to be the most susceptible. Indigenous domestic ruminants are usually more resistant to the disease. Wild animals could play a role as reservoir.
- Heartwater causes severe disease in cattle, sheep, and goats, with milder disease in some indigenous African breeds of sheep and goats, and inapparent disease in several

species of antelope indigenous to Africa. *Bos indicus* (Zebu) cattle breeds are in general more resistant than *Bos taurus* (European) breeds. Up to 80% of merino sheep may die, but the mortality rate can be only 6% in Persian or Afrikaner sheep. Angora and Saanen goats are also very susceptible to heartwater, while Creole goats in Guadeloupe show a significant level of resistance

- Heartwater has caused mortality in the African buffalo (*Syncerus caffer*) in some situations. Other species that have shown to be susceptible are the blesbok (*Damaliscus albifrons*), the black wildebeest (*Connochaetes gnu*), the eland (*Taurotragus oryx oryx*), giraffe (*Giraffe camelopardalis*), greater kudu (*Tragelaphus strepsiceros*), sable antelope (*Hippotragus niger*), sitatunga (*Tragelaphus spekii*), steenbok (*Raphicerus campestris*), and lechwe (*Kobus leche kafuensis*). It is believed that these species serve as reservoirs of heartwater and the disease in these animals is usually mild or undetectable.
- Deaths in springbok (*Antidorcas marsupialis*) in South Africa have been attributed to heartwater.
- A number of non-African ruminants are susceptible to heartwater experimentally and include the Timor deer (*Cervus timorensis*) and chital (*Axis axis*) of southern Asia, and white-tailed deer (*Odocoileus virginianus*) of North America. *Amblyomma maculatum* and *A. cajennense* ticks are experimentally proven vectors of heartwater, and are common parasites of the white-tailed deer in the southern United States.
- Rusa deer, white-tailed deer, springbok, chital, and timor deer, which are used in wildlife farming, seem to be the main wild ruminant species in which heartwater can have a significant economic impact.
- There is one report of an African elephant dying of heartwater, but this animal was also infected with anthrax. Other species suspected to be susceptible to heartwater but lacking in definitive proof are nilgai (*Boselaphus tragocamelus*), fallow deer (*Dama dama*), Himalayan tahr (*Hemitragus jemlahicus*), barbary sheep (*Ammotragus lervia*), mouflon (*Ovis aries*), blackbuck (*Antilope cervicapra*), and white and black rhinoceros.
- It was once believed that the guinea fowl and leopard tortoises were the nonruminant hosts of *E. ruminantium*, but recent data have confirmed that these species are not susceptible and do not transmit to vector ticks that usually feed on them. The scrub hare's susceptibility to infection is also not fully substantiated. Although the striped mouse and the multimammate mouse have been shown to be susceptible to *E. ruminantium*, they are not hosts of the vector ticks and are not believed to play a role in the epidemiology of heartwater. Some laboratory inbred strains of mice have been shown to be susceptible to *E. ruminantium* and have assisted in defining disease and immune mechanisms, but these are not indicated as important in disease maintenance.

Transmission

- Heartwater is transmitted transstadially by ticks of the genus Amblyomma, which are biological vectors of heartwater. Ticks become infected by feeding on acutely ill or subclinically infected animals. Of the 13 species capable of transmitting the disease, *A. variegatum* (tropical bont tick) is by far the most important because it is the most widespread. Other major vector species are the bont tick *A. hebraeum* (in southern Africa), *A. gemma* and *A. lepidum* (in Somalia, East Africa and the Sudan).
- *A. astrion* (mainly feed on buffalo) and *A. pomposum* (distributed in Angola, Congo [Dem. Rep. of the] and Central African Republic) are also natural vectors of the disease. Four other African ticks, *A. sparsum* (feed on reptiles and buffalo mainly), *A. cohaerans* (feed on African buffalo), *A. marmoreum* (adults occur on tortoises and immature stages on goats) and *A. tholloni* (adults feed on elephants) experimentally transmit heartwater.
- Three North American species of *Amblyomma* ticks also experimentally transmit heartwater: *A. maculatum* (the Gulf Coast tick), *A. cajennense* (the Cayenne tick) and *A. dissimile*, but none of these ticks has been incriminated so far in natural transmission of heartwater. A. maculatum is widely distributed in the eastern, southern, and western U.S., and feeds on ungulates (cattle, sheep, goats, horses, pigs, bison, donkeys, mules, white-tailed deer, sambar deer and axis deer), various carnivores, rodents and lagomorphs, marsupials, birds, and reptiles. A. maculatum was shown to be as efficient as A*. hebraeum*, and was susceptible to a wide range of *E. ruminantium* strains. *A. cajennense* has host preference similar to *A. maculatum* but is not as widely distributed and is a less efficient heartwater vector. *A. dissimile* feeds on reptiles and amphibians.
- *Amblyomma* ticks are three-host ticks whose life cycles may take from 5 months to 4 years to complete. Because the ticks may pick up the infection as larvae or nymphs and transmit it as nymphs or as adults, the infection can persist in the tick for at least 15 months. Infection does not pass transovarially. While transmission of heartwater can

be by adult and nymphal ticks in the field, in general adults prefer to feed on cattle and nymphs on sheep and goats.

- Cattle egrets have been implicated in the dispersal of *Amblyomma* ticks in the Caribbean.
- Heartwater can be transmitted vertically and through colostrum of carrier dams. Transmission can also occur by intravenous inoculation of blood, tick homogenates or cell culture material containing *E. ruminatium*.

Sources of the agent

- *Amblyomma* ticks fed on an infected vertebrate host.
- Whole blood or plasma of vertebrate host during the febrile reaction, but highest levels of agent occur during the second or third day of fever.
- Colostrum containing infected cells (reticulo-endothelial cells and macrophages) has been speculated.

Occurrence

Heartwater occurs in nearly all the sub-Saharan countries of Africa where *Amblyomma* ticks are present and in the surrounding islands: Madagascar, Reunion, Mauritius, Zanzibar, the Comoros Islands and Sao Tomé. The disease is also reported in the Caribbean (Guadeloupe, Marie-Galante and Antigua), from where it threatens the American mainland.

For more recent, detailed information on the occurrence of this disease worldwide, see the OIE World Animal Health Information Database (WAHID) Interface [http://www.oie.int/wahis/public.php?page=home] or refer to the latest issues of the World Animal Health and the OIE *Bulletin*.

DIAGNOSIS

The average incubation period in natural infections is 2–3 weeks, but can vary from 10 days to 1 month. The incubation period after intravenous blood inoculation is seven to 10 days in sheep and goats, and 10 to16 days in cattle. However the incubation is strongly dependent on the dose of elementary bodies inoculated as shown experimentally using in vitro cultivated *E ruminantium*. The outcome can range from 100% death with high doses to 0% death with low doses followed by protection of animals.

Clinical diagnosis

Heartwater occurs in four different clinical forms, determined by variations in host susceptibility, agent virulence and infective dose. Peracute disease is usually seen in Africa in non-native breeds of sheep, cattle and goats. Heavily pregnant cows are particularly susceptible to this form. Peracute disease is characterised by sudden death preceded by a brief interval of fever, severe respiratory distress, hyperesthesia, lacrimation and, in some breeds of cattle, severe diarrhoea. Terminal convulsions may be seen. This form of heartwater is relatively rare.

Acute disease is the most common form of heartwater in domesticated ruminants, and is seen in both non-native and indigenous cattle, sheep and goats. Animals with the acute form of heartwater usually die within a week.

- Disease begins with pyrexia, which may exceed 41°C within 1–2 days after onset. It remains high for 4–5 weeks with small fluctuations and drops shortly before death.
- Fever is followed by inappetence, sometimes listlessness, diarrhoea (particularly in cattle), and dyspnoea indicative of lung oedema.
- Nervous signs develop gradually, and are generally less pronounced in sheep and goats than cattle. The animal is restless, walks in circles, makes sucking movements and stands rigidly with tremors of the superficial muscles. Cattle may push their heads against a wall or present aggressive or anxious behaviour.
- In terminal stage the animal falls to the ground into lateral recumbency, pedalling and exhibiting opisthotonos, nystagmus, hyperaesthesia, chewing movements, and frothing at the mouth. The animal usually dies during or following such an attack.

On rare occasions, heartwater occurs as a subacute disease with prolonged fever, coughing and mild incoordination. CNS signs are inconsistent in this form. The animal either recovers or dies within 1 to 2 weeks.

- Mild or subclinical infections may be seen in young calves, lambs or kids; partially immune livestock; some indigenous breeds; and some wild ruminants. The only sign may be a transient fever.
- Morbidity is highly variable and depends on the degree of tick infestation, previous exposure to infected ticks, and level of acaricide protection.
- Once signs of the disease have developed, the prognosis is poor for non-native and exotic sheep, goats, and cattle. The mortality rate in non-native breeds of sheep and goats may be 80% or higher, in contrast to 6% in native breeds. In cattle, mortality of 60–80% is not uncommon.
- Recovery from heartwater infection usually results in complete immunity against homologous strains, although animals remain carriers of infection.

Lesions

The gross lesions in cattle, sheep, and goats are very similar. Heartwater derives its name from one of the prominent lesions observed in the disease, namely pronounced hydropericardium. The most common macroscopic lesions are hydropericardium, hydrothorax, pulmonary oedema, intestinal congestion, oedema of the mediastinal and bronchial lymph nodes, petechiae on the epicardium and endocardium, congestion of the brain, and moderate splenomegaly.

- Accumulation of straw-coloured to reddish fluid in the pericardium is more consistently observed in sheep and goats than in cattle.
- Hydropericardium, hydrothorax, ascites (mild), mediastinal oedema, and pulmonary oedema are common and result from increased vascular permeability.
- Oedema of the mediastinal and bronchial lymph nodes may occur.
- Froth in the trachea is often seen, reflecting terminal dyspnoea due to pulmonary oedema.
- Subendocardial petechial haemorrhages are usually present.
- Submucosal and subserosal haemorrhages may occur elsewhere in the body.
- Gross brain lesions are usually absent except for subtle swelling of the brain, which may result in conal herniation.
- Nephritis of varying degree, especially in Angora goats.
- Congestion and/or oedema of the abomasal folds are a regular finding in cattle, but less so in sheep and goats.

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.
- Accumulations of fluid similar to heartwater are also sometimes seen in heavy helminth infestations (haemonchosis).

Laboratory diagnosis

Samples

- Brain (cerebrum, cerebellum or hippocampus): Heartwater is often diagnosed in brain samples at necropsy. The best samples to collect are well vascularised portions of the brain such as the cerebrum, cerebellum or hippocampus. Brain tissue can be collected at necropsy by driving a large nail through the unopened skull, and aspirating a sample with a syringe. Another technique is to cut off the head and collect tissue through the foramen magnum with a curette. *E. ruminantium* colonies can be found for up to two days in brains stored at room temperature, and for up to 34 days in refrigerated brains. *E. ruminantium* can also be found in smears made from the intimae of large blood vessels.
- Whole blood in anticoagulant: 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 Terrestrial Manual*. Samples should be kept refrigerated and shipped with ice packs. PCR can also be attempted on brain, lungs, kidneys, and thoracic fluids.

- Serum: May be collected for serology, but false positive and false negative results may occur.
- *Amblyomma* ticks (adults and nymphs) if present should be placed in clean tubes either containing 70% alcohol for detection of *E. ruminantium* DNA sequences or in ventilated-top tubes to preserve the ticks' viability for xenodiagnosis.
- Set of tissues, including brain, in 10% buffered formalin.

Procedures

Identification of the agent

- Heartwater is often diagnosed by observing *E. ruminantium* colonies in the brain or intima of blood vessels. Brain smears are air dried, fixed with methanol and stained with Giemsa. *E. ruminantium* occurs as clumps of reddish-purple to blue, coccoid to pleomorphic organisms in the cytoplasm of capillary endothelial cells. These organisms are often found close to the nucleus, and may be in a ring or horseshoe. Colonies can be difficult or impossible to find in some animals that have been treated with antibiotics. Only a few colonies may be found in animals with peracute disease. The number of colonies in brains smears may also vary between strains with very rare colonies even with some very virulent strains. 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. *E. ruminantium* can also be detected in formalin-fixed brain sections using immunoperoxidase techniques.
- Polymerase chain reaction (PCR) techniques are available to reveal the presence of *E. ruminantium* in the blood of animals with clinical signs, in the tick vector, and to a lesser extent in the blood or bone marrow of carrier animals. PCR can detect the agent in the blood from just before the onset of fever to a few days after recovery, but detection in carrier animals is inconsistent. PCR can also be attempted on other target organs, such as brain, lungs, kidneys, and thoracic fluids. Apart from diagnosis, PCR is widely used for research on the *E. ruminantium* genome and for epidemiological studies. Less sensitive DNA probes are also available.
- Heartwater can also be diagnosed by isolating *E. ruminantium* from the blood. However, culture is time-consuming, and other diagnostic techniques may be preferred. *E. ruminantium* can be grown in many primary ruminant endothelial cells or endothelial cell lines. In cultures, the organism is identified by microscopic examination, or by immunofluorescence/immuno-peroxidase staining. In some cases, heartwater may be diagnosed by inoculating fresh blood into a susceptible sheep or goat.

Serological tests

- Serological tests available include indirect fluorescent antibody tests, enzyme linked immunosorbent assays (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.
- One ELISA uses a recombinant antigen expressed as a partial fragment of the recombinant major antigenic protein 1 (MAP1) antigens –the MAP1-B ELISA. This test has dramatic improvement in specificity compared with previous tests. Although more specific, it still detects cross-reacting antibodies to other *Ehrlichia* organisms (*E. canis*, *E. chaffeensis*, *E. ewingii* and agents which are yet to be fully characterised). Hence, definitive proof of heartwater must rely on epidemiological evidence and additional molecular testing. This ELISA has made the interpretation of serological results more reliable in regions where *Ehrlichia* infections occur in ruminants. It can help to monitor experimental infections and to measure the immune response of immunised animals, whose preimmunisation serological history is known. Serology has very limited diagnostic use as clinically infected animals remain seronegative during the febrile reaction and seroconvert after they recover from the infection.
- Serology is also not an effective import test. Prior to importation of animals from a heartwater endemic region, it is important to study the epidemiological data to try to establish that the herd and the resident ticks are not infected; in addition repeated PCR testing should be carried out to demonstrate that the pathogenic agent is not present in the herd.
- Serological diagnosis of heartwater is subjective and should be used only as a tool of investigation rather than for definitive diagnosis. Definitive diagnosis should be by demonstration of the organism on a smear, or by PCR amplification using the pSC20 nested-PCR assay and corroborated by isolation of *E. ruminantium* in endothelial cell culture.

For more detailed information regarding laboratory diagnostic methodologies, please refer to Chapter 2.1.6 Heartwater in the latest edition of the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* under the heading "Diagnostic Techniques".

PREVENTION AND CONTROL

- As *E. ruminantium* cannot survive outside a living host for more than a few hours at room temperature, heartwater is usually introduced into free areas by infected animals, including subclinical carriers, or by 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.
- Ticks may be carried into a country on illegally imported animals or migrating birds.
- Outbreaks are usually controlled with quarantines, euthanasia of infected animals and tick control.
- During an outbreak, ticks should not be allowed to feed on infected animals.
- Iatrogenic transfer of blood between animals must also be avoided.
- In endemic regions, heartwater can be prevented by tick control and vaccination.
- Animals moved into endemic areas may be protected by prophylactic treatment with tetracycline.
- Vaccination currently consists of infection with a live *E. ruminantium* strain, then treatment with antibiotics when a fever develops. Alternatively, the vaccine may be given to young kids or lambs during their first week of life, or to calves less than 5 to 8 weeks of age; young animals possess a degree of non-specific resistance to infection, and do not always require treatment. Vaccination does not protect animals from all field strains, and revaccination is risky due to the possibility of anaphylactic reactions. Inactivated and attenuated vaccines have proved to be efficient in controlled conditions but they do not overcome the strain diversity problem. Improved vaccines are in development.
- Intensive tick control may increase the susceptibility of animals to heartwater, because it eliminates the immune boosting effect of persistent exposure to small doses of organisms.
- In endemic areas, animals with heartwater can be treated with antibiotics.
- Tetracycline (oxytetracycline at 10 mg/kg or doxycycline at 2 mg/kg) 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.
- Vector control measures aimed at eradication of *Amblyomma* ticks by acaricide treatment of cattle and small ruminants has been successful in the context of small islands in the Caribbean but is not achievable in most situations and even not recommended. In endemic areas of Africa, tick levels are now allowed to remain at levels high enough to permit reinfection of immune animals to boost the immunity and develop endemic stability.

Medical prophylaxis

- No commercial vaccines are available at present. The only method of immunisation commercially available against heartwater remains the ‘infection and treatment’ method using infected blood followed by treatment of reacting animals with tetracycline. This method is still in use in several areas, but it is likely to be replaced soon by preparations using attenuated or inactivated organisms, which have given promising research results.
- A first generation vaccine consisting of inactivated purified elementary bodies of *E. ruminantium* emulsified in Montanide ISA 50 adjuvant has given good results in experimentally controlled conditions and has demonstrated significant protection in the field. Three different isolates (Senegal, Gardel and Welgevonden) have been attenuated and shown to confer good protection, and significant protection has also been obtained using DNA vaccination. However, none of these new experimental vaccines has been fully validated under field conditions. Field trials have revealed that antigenic diversity is important in formulating effective vaccines, and further investigations are critical for the delivery of any vaccine in the field.

Inactivated vaccines

- Inactivated vaccines using the Gardel strain and subsequently other strains have been produced in bioreactors for an industrial production following the development of the whole production process. The vaccine produced has the same efficacy as the initial laboratory-produced experimental vaccine and a production cost calculated at around 0.1 € per dose which becomes acceptable.

- The Mbizi strain inactivated vaccine is being developed commercially by Onderstepoort Biological Products in South Africa.
- These inactivated vaccines do not prevent infection but do prevent or reduce death of vaccinated animals when exposed to live virulent challenge. They confer protection for more than one year. The advantage is that several field strains can be incorporated to make the vaccine more widely cross-protective.
- A major challenge remains the characterisation of the extent of strain diversity in a region to be covered by an appropriate formulation of the vaccine. This knowledge will also be essential for new generation vaccines that will be developed in the future.

Live attenuated vaccines

- Infection of ruminants with live *E. ruminantium* strains induces a strong long-lasting protection against an homologous isolate. This is the basis for infection and treatment using virulent isolates.
- Isolates of attenuated virulence that do not necessitate treatment of animals would be ideal but a limited number of such attenuated isolates are available.
- An attenuated Senegal isolate has been obtained and shown to confer 100% protection against an homologous lethal challenge, but very poor protection against a heterologous challenge.
- The Gardel isolate, which gives a significant level of cross-protection with several isolates (although far from complete), has also been attenuated.
- Recently, a third isolate named Welgevonden from South Africa has been attenuated and shown to confer complete protection against four heterologous isolates under experimental conditions.
- The main drawback of attenuated vaccines is their extreme labiality, which necessitates their storage in liquid nitrogen and their distribution in frozen conditions. In addition, they have to be administered intravenously.

Recombinant vaccines

- Several reports show partial protection of mice using map1 DNA vaccination and an improvement of protection by vaccination following a prime (plasmid) – boost (recombinant MAP1) protocol. However, protection of ruminants has never been demonstrated using this strategy.
- In opposition, significant protection of sheep was reported against homologous and heterologous experimental challenge following plasmid vaccination using a cocktail of four ORFs (open reading frames) from the 1H12 locus in the *E. ruminantium* genome. No further results have been described since then.
- Recombinant vaccines will probably not be available in the near future.

For more detailed information regarding vaccines, please refer to Chapter 2.1.6 Heartwater in the latest edition of the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* under the heading "Requirements for Vaccines".

PUBLIC HEALTH

E. ruminantium is not thought to be zoonotic, but recently, positive PCR results for this organism were obtained in three fatal cases of human ehrlichiosis in South Africa. Two cases occurred in children with encephalitis, vasculitis of the brain, and pulmonary oedema. Clinical details were not available for the other case. *E. ruminantium* was not proven to be the cause of death in any of the three people, and it remains to be determined whether this organism can cause human disease. However it should be kept in mind that *E ruminantium* infects easily human endothelial cells in vitro and that immature Amblyomma ticks (potentially infected) can bite humans. It has thus a zoonotic potential.

For more detailed information regarding safe international trade in terrestrial animals and their products, please refer to the latest edition of the OIE *Terrestrial Animal Health Code*.

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The OIE will periodically update the OIE Technical Disease Cards. Please send relevant new references and proposed modifications to the OIE Scientific and Technical Department (scientific.dept@oie.int). Last updated October 2009.