

# Heartwater

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**Heartwater**

Cowdriosis, Malkopsiekte,  
Pericardite Exsudative Infectieuse,  
Hidrocarditis Infeciosa,  
Idropericardite Dei Ruminanti

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**Overview**

- Organism
- Economic Impact
- Epidemiology
- Transmission
- Clinical Signs
- Diagnosis and Treatment
- Prevention and Control
- Actions to Take



Center for Food Security and Public Health, Iowa State University, 2011

In today's presentation we will cover information regarding the organism that causes heartwater and its epidemiology. We will also talk about the economic impact the disease has had in the past and could have in the future. Additionally, we will talk about how it is transmitted, the species it affects, clinical signs, necropsy findings, and diagnosis and treatment of the disease. Finally, we will address prevention and control measures for the disease, as well as actions to take if Heartwater is suspected. [Photo of *Amblyomma variegatum* (the tropical bont tick), from Mat Pound at USDA, ARS.]


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**The Organism**

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***Ehrlichia ruminantium***

- Anaplasmataceae
  - Cytoplasm of capillary endothelial cells of infected animals
  - Coccoid, occasional rings
  - Clumps of a few to several thousand organisms
  - Red/purple to blue with Giemsa stain



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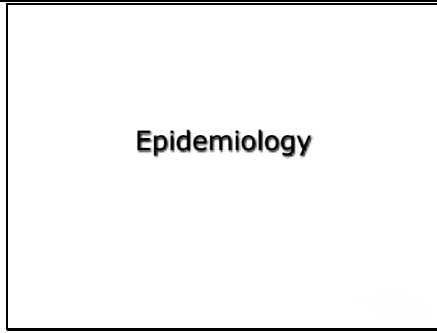
*Ehrlichia* (formerly *Cowdria*) *ruminantium* is a rickettsial bacterium (family Anaplasmataceae, order Rickettsiales). This organism is pleomorphic, measuring from 400 to more than 1,000 nm diameter. *E. ruminantium* has a high level of genomic plasticity. Gene segments are often deleted or inserted, and genes may be disrupted. Several different genotypes can co-exist in a geographic area, and may recombine to form new strains. The organism is an obligatory intracellular parasite and appears as clusters of reddish-purple to blue stained granules within the cytoplasm of infected endothelial cells when stained with Giemsa. The organism initially reproduces in macrophages, then invades and multiplies in endothelial cells, especially in the brain of ruminants. It cannot survive outside a living host for more than a few hours at room temperature. (Photo: Brain smear from a goat showing colonies of *C. ruminantium* as the granular blue areas in the cytoplasm of the capillary endothelial cells.  
From [www.vet.uga.edu/vpp/gray\\_book02/images/061.php](http://www.vet.uga.edu/vpp/gray_book02/images/061.php))

## Heartwater

S l i d e  5	<p style="text-align: center;"><b>Importance</b></p>	<p>Heartwater is a threat to the U.S. for several reasons. There is a risk of introduction of the disease through infected ticks or imported domestic animals or wildlife. A carrier state of the disease has been discovered in several wild animal species that have been imported to the U.S. Two tick species indigenous to the U.S. have been shown experimentally to serve as vectors for the organism responsible for causing heartwater.</p>
S l i d e  6	<p style="text-align: center;"><b>History</b></p> <ul style="list-style-type: none"><li>• 1830: South Africa – sheep</li><li>• 1898: Transmissibility</li><li>• 1900: Tropical bont tick vector</li><li>• 1925: Caused by Rickettsial agent</li><li>• 1980: Found in Western Hemisphere</li><li>• 1992 and 1997: Florida-imported vector ticks</li></ul> <small>Center for Food Security and Public Health, Iowa State University, 2011</small>	<p>The first historic identification of heartwater was made in sheep in South Africa in the 1830s. In 1898, heartwater was found to be transmissible experimentally by inoculation of blood from diseased to susceptible animals. The tropical bont tick (<i>Amblyomma variegatum</i>) was confirmed as a vector of the disease in South Africa in 1900. The rickettsial causative agent was found in the tissues of infected animals and ticks by Cowdry in 1925. In 1947, the rickettsial organism responsible for heartwater disease was named <i>Cowdria ruminantium</i>, after Cowdry (later changed to <i>Ehrlichia ruminantium</i>). The first reported occurrence of the disease in the Western hemisphere was in 1980 in Guadeloupe, one of the Caribbean Islands. Since then it has gradually spread to as far north as Puerto Rico, and southwards to Barbados and St. Vincent. The potential for the tropical bont tick, and thereby heartwater, to be introduced into the U.S. was demonstrated in 1992 when a cattle egret, banded in Guadeloupe (Caribbean), was found in the Florida Keys. In 1997, the same tick species was found on a traveler to the Caribbean upon her return to Florida.</p>
S l i d e  7	<p style="text-align: center;"><b>Economic Impact</b></p> <ul style="list-style-type: none"><li>• Zimbabwe<ul style="list-style-type: none"><li>– US \$5.6 million annual losses</li><li>– Acaricide, milk loss, treatment cost</li></ul></li><li>• Serious threat to the United States<ul style="list-style-type: none"><li>– Caribbean Islands with infected ticks</li><li>– Migratory cattle egrets</li><li>– Susceptible cattle and deer population</li></ul></li><li>• 40% to 100% death in U.S. expected</li></ul> <small>Center for Food Security and Public Health, Iowa State University, 2011</small>	<p>Heartwater is a serious constraint to livestock development in much of sub-Saharan Africa. In an assessment of the economic impact of heartwater and its control in Zimbabwe (Preventative Veterinary Medicine 1999;39:173-189), the estimated total annual national losses due to heartwater were U.S. \$5.6 million. Losses in commercial systems were 25 times greater than losses in the communal system. The greatest components of economic loss were acaricide costs (76%), followed by milk loss (18%), and treatment cost (5%). Heartwater is a serious threat to the United States considering the presence of the disease in the Caribbean with its proximity to the southern coast of the United States. Migratory birds, especially cattle egrets (<i>Bubulcus ibis</i>), have been demonstrated to fly from the Caribbean to Florida. Additionally, our ruminant populations are naïve, and therefore more susceptible, to the severe form of the disease. It has been estimated that between 40% and 100% mortality in the cattle population will be observed if heartwater enters the United States.</p>

## Heartwater


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### Geographic Distribution

- Sub-Saharan Africa
- The Caribbean Islands
  - Puerto Rico, Guadeloupe, Barbados, St. Vincent, Antigua, Marie, Galante
- Not reported in Asia
- Tick introduction into U.S. possible



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Heartwater occurs where its tick vectors are present. The disease is endemic in most of Africa south of the Sahara, as well as in surrounding islands such as Madagascar, and in the Caribbean. Carrier wildlife in these locations sustains the disease in nature. To date, heartwater has never been reported in Asia, despite the presence of many species of *Amblyomma* tick.

Heartwater is readily introduced into new regions in infected animals or ticks. Known and potential host ticks are widely distributed, and can be found on a variety of animals including reptiles. On at least one occasion, leopard tortoises and African spurred tortoises imported into Florida were found to be carrying infected ticks.

Jan jun 2010

[http://web.oie.int/wahis/public.php?page=disease\\_status\\_map&disease\\_type=Terrestrial&disease\\_id=20&disease\\_category\\_terrestrial=-1&empty=999999&disease\\_category\\_aquatic=-1&disease\\_serotype=0&sta\\_method=semesterly&selected\\_start\\_year=2010&selected\\_report\\_period=1&selected\\_start\\_month=1&page=disease\\_status\\_map&date\\_submit=OKs](http://web.oie.int/wahis/public.php?page=disease_status_map&disease_type=Terrestrial&disease_id=20&disease_category_terrestrial=-1&empty=999999&disease_category_aquatic=-1&disease_serotype=0&sta_method=semesterly&selected_start_year=2010&selected_report_period=1&selected_start_month=1&page=disease_status_map&date_submit=OKs). Two indigenous tick species in the United States, *A. maculatum* and *A. cajennense*, have been shown experimentally to be capable of serving as vectors for *E. ruminantium*.

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### Morbidity/Mortality

- Susceptible ruminants
- Morbidity: Approaches 100%
- Mortality:
  - 6% in Persian or Afrikander sheep
  - 60% in Cattle
  - 80% in Merino sheep and Angora goats

Center for Food Security and Public Health, Iowa State University, 2011

Untreated non-native cattle, sheep, and goats often have morbidity rates approaching 100%. Death rates of 80% have been recorded in Merino sheep and Angora goats. Persian and Afrikander sheep are more resistant to heartwater, with a mortality rate of 6%. Creole goats in Guadeloupe are also resistant. *Bos indicus* breeds of cattle tend to be more resistant than *Bos taurus*.

Most infections in wild ruminants appear to be subclinical or mild, but high mortality rates have been reported in lechwe introduced into endemic areas, and in experimentally infected white-tailed deer. Occasional cases of heartwater have also been reported in other wild ruminants in Africa. Heartwater was suspected to be the cause of death of a dromedary, but this was not proven.

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## Transmission

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### Animal Transmission

- Vector-borne
- *Amblyomma* ticks
- Larvae and nymphs infected
  - From infected animals
- Animal inoculation
  - Infected nymphs and adult ticks
  - Mammals, birds, and reptiles




Center for Food Security and Public Health, Iowa State University, 2011

Heartwater is primarily transmitted by ticks of the genus *Amblyomma* (Family Ixoidae). At least twelve species of *Amblyomma* ticks have been shown to transmit *Ehrlichia ruminantium*. *A. variegatum* (tropical bont tick) is the most important transmitter of heartwater (pictured above). These three-host ticks can become infected during larval or nymphal stages and transmit the organism to the subsequent life-cycle stage (transstadial transmission). Transovarial passage is not significant in the epidemiology of heartwater, and may not occur. (infected female ticks do not transfer the rickettsia to offspring). The life cycle of these ticks can take from 1-4 years. The immature stages feed on a wide variety of livestock, wild ungulates, ground birds, small mammals, reptiles, and amphibians. Ticks acquire the infection by feeding on acutely ill or subclinically infected animals. Thereafter, they probably retain the infection for life. Infected sheep and cattle remain infective to ticks for about 6 to 8 months, respectively. Some wild ruminants remain infective for 6 months; others can become asymptomatic carriers. (Photo from The Gray Book at [www.vet.uga.edu/vpp/gray\\_book02](http://www.vet.uga.edu/vpp/gray_book02))

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### Animal Transmission

- Cow to calf transmission
  - Via colostrum
- Tick spread
  - Cattle egret
  - Helmeted guinea fowl
- Natural reservoirs
  - Blesbok
  - Wildebeest



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In endemic areas, there has been evidence of transmission of *E. ruminantium* from infected cows to their calves through colostrum. Wild ruminants, such as blesbok (*Damaliscus dorcas phillipi*) and black wildebeest, as well as helmeted guinea fowl, the leopard tortoise (*Geochelone pardalis*), and possibly the scrub hare have been shown to harbor *E. ruminantium* subclinically for long periods and play a role as a source of infection for ticks. Cattle egrets have become established in many regions where heartwater is endemic, and have been implicated in the spread of the disease.

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## Animals and Heartwater

## Heartwater

S 1 i d e 1 5	<b>Affected Species</b>	<p>Heartwater causes severe disease in cattle, sheep, goats, and water buffalo; mild disease in some indigenous African breeds of sheep and goats; and inapparent disease in several species of antelope indigenous to Africa, such as blesbok, wildebeest, eland, and springbok. Most wildlife species appear to carry the organism asymptotically, but serious illness has been reported in lechwe moved into endemic areas, as well as in experimentally infected white-tailed deer; mortality was high. <i>Amblyomma maculatum</i>, an experimentally proven vector of heartwater, is a common parasite of white-tailed deer in the U.S. Several non-ruminant hosts have been shown to be carriers of <i>E. ruminantium</i>; these include guinea-fowl, leopard tortoises, and possibly the scrub hare.</p>
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- Severe disease
    - Cattle, sheep, goats, water buffalo
    - White-tailed deer (experimentally)
    - Lechwe
  - Mild disease
    - Indigenous African breeds of sheep and goats
  - Inapparent disease
    - Blesbok, wildebeest, eland, springbok
- Center for Food Security and Public Health, Iowa State University, 2011


S 1 i d e 1 6	<b>Clinical Signs</b>	<p>Clinical signs and lesions of heartwater are representative of injury to the vascular endothelium and the resulting increase in vascular permeability. The incubation period ranges from 14-28 days, typically being shorter in sheep and goats than in cattle. In experimentally inoculated (i.v.) ruminants, clinical signs manifest quicker [7-10 days in sheep and goats, 10-16 days in cattle]. Disease can be peracute (rare), acute (most common), subacute (rare), or mild/subclinical (calves). Severity of disease is determined by various strains of the heartwater agent and animal susceptibility.</p>
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- Incubation period: 14 to 28 days
    - Experimental intravenous inoculation
      - Sheep and goats: 7 to 10 days
      - Cattle: 10 to 16 days
  - Four forms of disease
    - Peracute (rare)
    - Acute (most common)
    - Subacute (rare)
    - Mild or subclinical
- Center for Food Security and Public Health, Iowa State University, 2011

S 1 i d e 1 7	<b>Clinical Signs: Acute</b>	<p>The most common form of heartwater is the acute form. This is seen in both non-native and indigenous domestic ruminants. Animals develop a sudden high fever, loss of appetite, depression, and respiratory distress and tachypnea. Some animals, particularly cattle, may also develop diarrhea. Nervous disorders can soon follow and be seen as excessive chewing movements, incoordination, head tilting upwards, overly rigid posture, and walking with a high stepping gait. Some animals may have convulsions. Galloping movements and opisthotonus are commonly seen before death, which generally occurs within one week.</p>
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- Most common form
  - Sudden fever (107°F)
  - Inappetence, depression
  - Tachypnea, respiratory distress
  - Nervous signs
    - Chewing movements, eyelid twitching, tongue protrusion, circling, paddling
  - Death in 1 week
- Center for Food Security and Public Health, Iowa State University, 2011

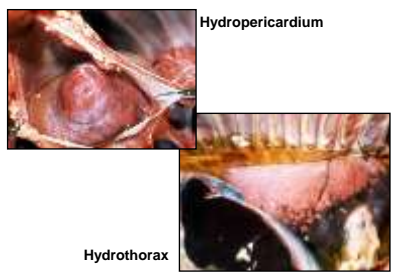
S 1 i d e 1 8	<b>Clinical Signs: Subclinical</b>	<p>The mild form of the disease is called “heartwater fever”. It is present in some affected regions among indigenous domestic breeds with resistance or partial immunity to heartwater, young ruminants (calves, lambs, and kids), and native antelope species and some other wild ruminants. The only symptom may be a transient fever. (Photo: wildebeest)</p>
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- “Heartwater fever”
  - Breeds with resistance or partial immunity
  - Asymptomatic
  - Transient fever
- 
- Center for Food Security and Public Health, Iowa State University, 2011

## Heartwater

S 1 i d e  1 9	<b>Clinical Signs: Other Forms</b> <ul style="list-style-type: none"><li>• Peracute<ul style="list-style-type: none"><li>– Sudden death</li><li>– Fever, severe respiratory distress, convulsions, ± severe diarrhea</li></ul></li><li>• Subacute<ul style="list-style-type: none"><li>– Prolonged fever, coughing, edema of lungs, mild incoordination</li><li>– Recovery or death in 1 to 2 weeks</li></ul></li></ul> <small>Center for Food Security and Public Health, Iowa State University, 2011</small>	<p>There are two other forms of heartwater that are rarely seen. The peracute form of heartwater is rare and typically occurs in non-native breeds of cattle, sheep, and goats introduced into an enzootic area. Heavily pregnant cows are especially prone to develop the peracute form of the disease. Sudden death is the typical manifestation for this form. Clinical signs seen prior to death may include fever, severe respiratory distress, and convulsions. Additionally, some breeds of cattle (Jerseys and Guernseys) may develop severe diarrhea. Clinical signs of the subacute form include prolonged fever and coughing due to edema of the lungs. Animals may show mild incoordination and will either recover in 1-2 weeks or succumb to death.</p>
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S 1 i d e  2 0	<b>Post Mortem Lesions</b> <ul style="list-style-type: none"><li>• Hydropericardium</li><li>• Hydrothorax</li><li>• Ascites</li><li>• Pulmonary and mediastinal edema</li><li>• Petechiae and ecchymoses<ul style="list-style-type: none"><li>– Mucosal and serosal surfaces</li><li>– GI tract, esp. abomasum</li></ul></li><li>• Congestion and edema in the brain</li></ul> <small>Center for Food Security and Public Health, Iowa State University, 2011</small>	<p>The characteristic post-mortem lesion for heartwater is hydropericardium with straw-to-red colored pericardial fluid; the reason for which the name ‘heartwater’ was derived. It is more consistently found in sheep and goats than in cattle. Other common lesions include hydrothorax, mediastinal and pulmonary edema and congestion, splenomegaly, petechiae and ecchymoses on mucosal and serosal surfaces, and occasionally hemorrhage of the gastrointestinal tract, especially the abomasum. Additionally, edematous lymph nodes (the mediastinal and bronchial in particular) may be noted, as well as congestion and meningeal edema in the brain.</p>
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S 1 i d e  2 1	 <small>Center for Food Security and Public Health, Iowa State University, 2011</small>	<p>Hydropericardium (top photo) and excessive fluid in the thoracic cavity with pulmonary edema (bottom photo). Note the distended interlobular septa. (Photos from The Gray Book at <a href="http://www.vet.uga.edu/vpp/gray_book02">www.vet.uga.edu/vpp/gray_book02</a>)</p>
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S 1 i d e  2 2	<b>Differential Diagnosis</b> <ul style="list-style-type: none"><li>• Anthrax (peracute; sudden death)</li><li>• Acute form<ul style="list-style-type: none"><li>– Rabies</li><li>– Tetanus</li><li>– Meningitis or encephalitis</li><li>– Babesiosis</li><li>– Cerebral trypanosomiasis</li><li>– Theileriosis</li><li>– Poisons</li></ul></li></ul> <small>Center for Food Security and Public Health, Iowa State University, 2011</small>	<p>Anthrax should be considered as a differential for the peracute form of heartwater (i.e., sudden death). Other diseases to consider with the acute form of heartwater include rabies, tetanus, bacterial meningitis or encephalitis, babesiosis, anaplasmosis, cerebral trypanosomiasis, or theileriosis. Additionally, poisonings from stychnine, lead, arsenic, ionophores and other myocardial toxins, organophosphates, chlorinated hydrocarbons, or some poisonous plants should be considered. Accumulations of fluid similar to heartwater are also sometimes seen in heavy helminth infestations.</p>
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## Heartwater

S 1 i d e 2 3	<h3>Sampling</h3> <ul style="list-style-type: none"><li>• Before collecting or sending any samples, the proper authorities should be contacted</li><li>• Samples should only be sent under secure conditions and to authorized laboratories to prevent the spread of the disease</li></ul> <small>Center for Food Security and Public Health, Iowa State University, 2011</small>	<p>Before collecting or sending any samples from animals with a suspected foreign animal disease, the proper authorities should be contacted. Samples should only be sent under secure conditions and to authorized laboratories to prevent the spread of the disease.</p>
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S 1 i d e 2 4	<h3>Diagnosis</h3> <ul style="list-style-type: none"><li>• Clinical signs<ul style="list-style-type: none"><li>- Fever, respiratory distress, sudden death</li><li>- Presence of <i>Amblyomma</i> ticks</li></ul></li><li>• Laboratory Tests<ul style="list-style-type: none"><li>- Microscopic identification organism</li><li>- PCR, IFA, ELISA</li></ul></li></ul>  <small>Center for Food Security and Public Health, Iowa State University, 2011</small>	<p>Heartwater should be suspected in animals with the typical clinical signs, including fever, respiratory distress, characteristic nervous symptoms, and sudden death, as well as characteristic post mortem lesions. The presence of the vector <i>Amblyomma</i> species would be additional evidence for suspicion. Diagnosis of heartwater can be done by identification of <i>E. ruminantium</i> in Giemsa-stained smears of cerebral or cerebellar gray matter, or the intima of blood vessels. PCR assays have provided more sensitive tests in both ticks and ruminants; however, detection in carrier animals is inconsistent. There is no adequate serodiagnostic test for heartwater at this time due to cross reaction with closely related <i>Ehrlichia</i> species. Other diagnostic tests include isolation and culture from blood samples, or animal inoculation. Heartwater carriers are difficult to detect. Rickettsial colonies are difficult to find in these animals, and animal inoculation may be unsuccessful except during the first few weeks after recovery. Carriers may sometimes be found by PCR or by feeding ticks on the animal and testing the ticks by PCR. Some carriers can be seronegative.</p>
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S 1 i d e 2 5	<h3>Treatment</h3> <ul style="list-style-type: none"><li>• Early stage<ul style="list-style-type: none"><li>- Oxytetracycline</li></ul></li><li>• Late stage<ul style="list-style-type: none"><li>- When neurological signs occur</li><li>- Usually futile</li></ul></li></ul>  <small>Center for Food Security and Public Health, Iowa State University, 2011</small>	<p>Tetracycline antibiotics (especially oxytetracycline) are very effective in the treatment of heartwater when used during the early, febrile stages of the disease. Treatment is usually ineffective if the first dose of oxytetracycline is not administered until after neurological signs appear. Animals often die before treatment can be administered.</p>
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S 1 i d e 2 6	<h3>Heartwater in Humans</h3>	<p><i>E. ruminantium</i> is not thought to be zoonotic. Recently, positive PCR results for this organism were obtained in three fatal cases of human ehrlichiosis. Two cases occurred in children with encephalitis, vasculitis of the brain, and pulmonary edema. Clinical details were not available for the other case. <i>E. ruminantium</i> 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.</p>
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**Prevention  
and Control**

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**Recommended Actions**

- IMMEDIATELY notify authorities
  - Federal
    - Area Veterinarian in Charge (AVIC)  
[http://www.aphis.usda.gov/animal\\_health/area\\_offices/](http://www.aphis.usda.gov/animal_health/area_offices/)
  - State
    - State veterinarian  
<http://www.usaha.org/StateAnimalHealthOfficials.pdf>
- Quarantine

Center for Food Security and Public Health, Iowa State University, 2011

If heartwater infection is suspected, state or federal authorities should be notified immediately. Animals with suspected heartwater should be isolated, and the farm should be quarantined until definitive diagnosis is determined.

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**Recommended Actions**

- Quarantine
  - All wild ruminants imported from Africa
    - PCR for *E. ruminantium*
  - All wild ungulates, birds, and reptiles imported to U.S.
    - Examine for ticks

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*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. Quarantine results are forwarded to the Veterinary Officer-In-Charge at Federal and State levels.

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**Prevention**

- Tick control program
  - Acaricides
- Importation control and monitoring
  - Infected wildlife or ticks
- The Caribbean *Amblyomma* Program
  - English and Dutch-speaking islands
- POSEIDOM Vétérinaire Programme
  - French-speaking islands

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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. Preventative measures for heartwater include implementation of an effective tick control program, as well as regular inspection of animals and pastures for ticks. Elimination of the vector can be achieved through the use of acaricides; however, acaricide resistance may develop. In endemic areas, 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. 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.

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**Vaccination**

- Attenuated *E. ruminantium*
  - BALL3-strain serial passage
  - Confers solid immunity
- Universally effective vaccine not developed

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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 5-8 weeks of age; young animals possess 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. The only commercial vaccine available is made from the blood of sheep infected with live *E. ruminantium* organisms BALL3-strain. The vaccine is administered intravenously into the jugular vein at a dosage of 3 ml for cattle, sheep, and goats, irrespective of age or size. Improved vaccines are in development. Alternatively, animals moved into endemic areas may be protected by prophylactic treatment with tetracycline.

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**Additional Resources**

- World Organization for Animal Health (OIE)
  - [www.oie.int](http://www.oie.int)
- U.S. Department of Agriculture (USDA)
  - [www.aphis.usda.gov](http://www.aphis.usda.gov)
- Center for Food Security and Public Health
  - [www.cfsph.iastate.edu](http://www.cfsph.iastate.edu)
- USAHA Foreign Animal Diseases ("The Gray Book")
  - [www.usaha.org/pubs/fad.pdf](http://www.usaha.org/pubs/fad.pdf)

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