Bovine Babesiosis

Tick Fever,
Cattle Fever,
Texas Fever,
Piroplasmosis,
Redwater

Last Updated: August 2018

Importance

Bovine babesiosis is a tick-borne parasitic disease that results in significant morbidity and mortality in cattle. The economic losses can be considerable, especially when animals with no immunity are moved into an endemic area. Three species of *Babesia* cause most clinical cases in cattle: *Babesia bovis* and *B. bigemina* are widespread in tropical and subtropical regions, while *B. divergens* circulates in parts of Europe and possibly in North Africa. Bovine babesiosis can be managed and treated, but the causative organisms are difficult to eradicate. The United States eradicated *B. bovis* and *B. bigemina* from most of the country by eliminating its tick vectors in an intensive campaign that took 40 years. Currently, these ticks persist only in a quarantine buffer zone between the U.S. and Mexico. Reintroduction is a significant threat; the tick vectors have been detected periodically outside this buffer zone, and acaricide resistance is a growing issue for control. Most cattle *Babesia* do not seem to affect humans; however, *B. divergens* can cause rapidly progressing, life-threatening hemolytic anemia in people who have had splenectomies.

Etiology

Babesia are protozoa in the family Babesiidae, order Piroplasmida. Bovine babesiosis is usually caused by *Babesia bovis, B. bigemina* and *B. divergens. B. major, B. ovata, B. occultans, B. jakimovi* and some unnamed organisms also infect cattle, but seem to be much less virulent. Little has been published about *B. jakimovi*, which was discovered in Russia and does not appear in many descriptions of *Babesia*.

B. divergens-like organisms

B. divergens belongs to the *Babesia divergens*/*B. odocoilei* complex, which also contains some closely-related, named or unnamed organisms that circulate in wildlife and do not seem to affect cattle. Some of these organisms are very difficult to distinguish from *B. divergens*. For example, the deer parasite *B. capreoli* does not infect cattle, but it can be amplified by PCR tests for *B. divergens*, and the sequence of a commonly amplified segment was found to be almost identical except at 4 specific sites. Because many studies do not include animal tests and detailed genetic analyses, the identity of some organisms remains unclear, and they are often called *B. divergens*-like. However, this does not imply that they are probably *B. divergens*. For instance, *B. divergens*-like organisms were detected in wild rabbits on the East Coast of the U.S., and later found to be a different organism that does not infect cattle.

Species Affected

Cattle are the primary hosts and reservoirs for *B. bovis*, *B bigemina* and *B. divergens*. These organisms are sometimes detected in other animals by PCR, but nucleic acids alone do not prove that the animal is susceptible: PCR tests might detect organisms inoculated by ticks that fed recently on the animal, and they can sometimes also amplify closely-related species of *Babesia*. Nucleic acids of *B. divergens* have been definitively identified in some naturally infected reindeer (*Rangifer tarandus*), and this species was also susceptible to experimental inoculation. Sheep were experimentally infected with this organism in a recent study, although not all individual sheep were susceptible. Previous reports had suggested that sheep could not be infected with *B. divergens* unless the spleen, which is important in controlling *Babesia*, is removed. Other ungulates, such as deer and mouflon (*Ovis musimon*), only seem to be susceptible if they are splenectomized, and most reports of PCR-positive wild species are probably caused by a closely-related organism, such as *B. capreoli.* Experimentally infected, splenectomized non-human primates (including chimpanzees, *Pan troglodytes* and rhesus macaques, *Macaca mulatta*) become ill, but spleen-intact primates were unaffected. Mongolian gerbils are the only laboratory rodents that are readily infected with *B. divergens* without removing the spleen.

B. bovis and *B bigemina* have been detected by PCR in water buffalo (*Bubalus bubalis*), but clinical cases in this species are normally caused by *B. orientalis*, which does not infect cattle. Nucleic acids of *B. bovis* have been found in a few wild African buffalo (*Syncerus caffer*), and experimental infections with *B. bigemina* were established in African buffalo and American bison (*Bison bison*). Several PCR-based

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studies have detected *B bovis* and *B. bigemina* in wild white-tailed deer (*Odocoileus virginianus)*. However, white-tailed deer could not be experimentally infected with *B. bovis* in a recent study. *B bovis* and *B. bigemina* have also been found by PCR in nilgai antelope (*Boselaphus tragocamelus*), pampas deer (*Ozotoceros bezoarticus*) and horses, and nucleic acids of *B. bigemina* were detected in roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*), wild boar (*Sus scrofa*), yaks (*Bos grunniens*), impala (*Aepyceros melampus*) and a greater kudu (*Tragelaphus strepsiceros*). Splenectomized gazelles (*Gazella soemmerring*) were experimentally infected with *B. bigemina*, but one splenectomized and one spleen-intact eland (*Taurotragus oryx*) were not susceptible.

Minor species

B. major, *B. ovata*, and *B. occultans* are primarily thought to infect cattle. Nucleic acids of *B. occultans* have also been reported in wild African buffalo, and *B. major* can cause clinical signs in experimentally infected American bison. *B. jakimovi* has been reported to infect cattle, roe deer, Asian elk (*Alces alces*), and reindeer.

Zoonotic potential

There is no indication that most cattle *Babesia* infect humans; however, *B. divergens* can cause serious illnesses in people who have had splenectomies. Whether this organism can cause mild or asymptomatic infections in people with an intact spleen is still debated. Clinical cases thought to be caused by *B. bovis* were reported in the past, but most or all of these organisms were probably *B. divergens* or other misidentified species.

Geographic Distribution

B. bovis and *B. bigemina* are mainly found in tropical and subtropical regions. Although there are some differences in their distribution, these two organisms have been reported from Asia, Africa, the Middle East, Australia, Central and South America, parts of southern Europe, and some islands in the Caribbean and South Pacific. In North America, *B. bovis*, *B. bigemina* and their tick vectors occur only in Mexico and a quarantine buffer zone in the U.S. along the Mexican border. However, suitable habitat for the tick vectors is present in the southern U.S., where they were formerly endemic.

B. divergens causes bovine babesiosis in parts of Europe, where it occurs as far south as Turkey. This organism was also detected in Tunisia, which led to the suggestion that it might be established in parts of North Africa. The usual vector for *B. divergens*, *I. ricinus*, can be found from Scandinavia to North Africa. However, this tick requires 80% humidity and only occurs in certain microenvironments such as the base of vegetation in forests, rough hill scrub and damp, low-lying land.

B. major has been reported from parts of Europe, Africa and Asia, and *B. ovata* from parts of Asia. *B. occultans* has been found in Africa (including North Africa) and the Balearic Islands (Spain), and there is some evidence for its existence in Turkey. A similar organism, *Babesia* sp. Kashi 2, occurs in China. Some authors suggest that *B. occultans* and *Babesia* sp. Kashi 2 may be the same species and that this organism is probably in widespread in Africa and Asia. *B. jakimovi* was found in Siberia.

Transmission

Babesia are transmitted by ticks. The major vectors for *B. bigemina* and *B. bovis* are *Rhipicephalus microplus* (formerly *Boophilus microplus*), and in some areas, *R. annulatus* (formerly *Boophilus annulatus*). *R. microplus* and *R. annulatus* are one-host ticks that complete their life cycle on a single host, and preferentially feed on cattle. Additional members of *Rhipicephalus* and some ticks in other genera have also been suggested as vectors in some regions. *Babesia* can be transmitted transovarially. They are stimulated to undergo their final maturation when an infected tick attaches to the host. *B. bovis* usually becomes infective within 2-3 days after larval ticks attach. It does not persist in *R. microplus* after the larval stage. *B. bigemina* matures approximately 9 days after larval attachment, and it is only transmitted by nymphs and adults.

Ixodes ricinus is the major vector for *B. divergens*. All three of its life stages are thought to be capable of transmitting this organism. *Haemaphysalis longicornis* transmits *B. ovata*, *while B. occultans* is thought to be transmitted by *Hyalomma marginatum*, *Hy. rufipes* and possibly other members of this genus. The vectors for *B. major* are thought to include *Haemaphysalis punctata* and possibly other members of this genus. *B jakimovi* might be transmitted by a member of the genus *Ixodes*.

Cattle that have recovered from acute babesiosis can remain asymptomatically infected, and recrudescence of parasitemia can occur at irregular intervals. Persistent infection with *B. divergens*, with periodic waves of parasitemia, was also detected in some experimentally infected sheep. *Babesia* can be transmitted directly between animals in blood, for instance during transfusions, and possibly when smaller amounts of blood are transferred on reused needles or field surgical instruments or by biting flies. Transplacental transmission has been demonstrated for *B. bovis* and *B. bigemina* in cattle, but seems to be infrequent.

Humans are thought to become infected with *B. divergens* in tick bites. Other species of zoonotic *Babesia* (e.g., *B. microti* of rodents*)* can be transmitted in blood transfusions, and may also infect the fetus *in utero* on rare occasions. One HIV-infected, splenectomized patient might have acquired *B. divergens* in a blood transfusion, but a tick bite was also plausible.

Disinfection

Disinfection is not important in the control of babesiosis. If needed, an agent effective against protozoa should be selected.

Infections in Animals

Incubation Period

Clinical signs usually appear 2-3 weeks after a bite from an infected tick. After inoculation with contaminated blood, the incubation period can be as short as 4-5 days for *B. bigemina* and 10-12 days for *B. bovis*.

Clinical Signs

Babesiosis is characterized by fever, which can be high, and varying degrees of hemolysis and anemia. Anemia may develop rapidly. The resulting clinical signs can include pale mucous membranes and increased respiratory and heart rates, as well as a decreased appetite, a drop in milk production, weakness, lethargy, and other signs related to anemia or fever, including abortions or temporarily decreased fertility in bulls. Jaundice is sometimes apparent, especially when the clinical signs are less acute, and hemoglobinuria and hemoglobinemia are common in animals infected with *B. bigemina*. *B. bovis* can cause additional clinical signs via changes in red blood cells (RBCs) that result in their accumulation in capillaries, including those of the brain. This can result in neurological signs (e.g., incoordination, teeth grinding, manic behavior), and may cause or contribute to other serious syndromes such as respiratory distress. *B. bigemina* and *B. divergens* do not cause similar changes in RBCs, and neurological signs are uncommon in cattle infected with these organisms. However, they may occur if anemia results in brain anoxia. "Pipestem" diarrhea is reported to be common in the early stages of babesiosis caused by *B. divergens*, from changes in intestinal and ruminal motility. Terminal recumbency, dehydration and constipation may occur in the late stages of babesiosis. In animals that survive, the anemic crisis generally passes within a week. The survivors may be weak and in reduced condition, although they usually recover fully.

The severity of babesiosis can vary considerably between individuals, and cattle younger than 9 months are usually infected without clinical signs. Mild illnesses, with mild fever, anorexia and an uneventful recovery, are also reported to be common in animals infected with *B. divergens*. A few congenitally infected calves were reported to have signs of babesiosis, including neurological signs. In one case, a clinically affected calf was born to a dam with no apparent history of babesiosis. Some calves seem to be infected *in utero* but asymptomatic at birth.

B. bovis and *B. bigemina* usually seem to infect water buffalo without clinical signs; however, some strains of *B. bovis* can cause a subclinical decrease in the hematocrit. American bison inoculated with *B. bigemina* had acute signs similar to those in cattle with severe babesiosis. Clinical babesiosis has been seen in naturally infected reindeer; however, it is not clear whether *B. divergens* or *B. capreoli*, which also infects reindeer, was responsible for these cases. Definitive identification of *B. divergens* has only been reported, to date, in asymptomatic animals. However, reindeer experimentally infected with this organism can become ill and may die. African buffalo experimentally infected with *B. bigemina* and sheep inoculated with *B. divergens* remained asymptomatic. Various splenectomized ungulates inoculated with *B. divergens* were also generally asymptomatic. Splenectomized non-human primates became severely ill after inoculation with this organism.

Minor species

B. major, *B. ovata* and *B. occultans* are mostly thought to cause mild illnesses or asymptomatic infections in cattle, but there are occasional reports of clinical cases. *B. major* has been implicated in anemia and hemoglobinuria, and it is thought to have been responsible for two fatal cases of babesiosis in Hungary, while *B. occultans* appears to have caused babesiosis in a herd of cattle in Italy. *B. ovata* may potentiate the development of anemia in cattle co-infected with *T. orientalis*, and it can cause clinical signs in experimentally infected, splenectomized cattle. Some authors have speculated that this organism might cause clinical babesiosis in animals that are immunocompromised from other causes.

Post Mortem Lesions Click to view images

The gross lesions of babesiosis are mainly related to intravascular hemolysis, anemia and jaundice. The mucous membranes are usually pale and may be icteric, and the blood can appear thin and watery. Icterus may also be observed in the omentum, abdominal fat and subcutaneous tissues. The spleen is markedly enlarged with a dark, pulpy, friable consistency. The liver may be enlarged and darkened or icteric, with a distended gallbladder containing thick, granular bile. The kidneys are usually dark red or black, and the urinary bladder often contains reddish–brown urine; however, the appearance of the urine is sometimes normal. The lungs occasionally show signs of pulmonary edema. Other organs including the heart and brain may have petechiae or ecchymoses or be congested, and the surface of the brain can look pink.

Diagnostic Tests

Babesiosis is often diagnosed by identifying the parasites in blood or tissue smears stained with Giemsa. Fluorescent dyes such as acridine orange can aid in parasite identification, and immunostaining techniques have been described. *B. bigemina* and *B. divergens* can be found in normal venous blood samples, but *B. bovis* is more likely to be recovered from capillary blood. Samples should be taken from capillaries in the ear or tail if the latter organism is suspected. At necropsy, recommended samples include the kidney, myocardium, liver and lung. The brain (cerebral

cortex) can also be sampled in animals with neurological signs. Diagnosis is unreliable if an animal has been dead for more than 24 hours, but parasites can sometimes be found in blood from the lower leg. Bovine *Babesia* are detected most easily in acutely infected animals; carriers can be difficult to identify with this technique. Treatment can clear the organisms rapidly from the circulation, although the animal remains ill from their effects.

Babesia are identified under oil immersion. The World Organization for Animal Health (OIE) recommends a 10x eyepiece and 100x objective lens, at a minimum. Slides for thin blood films are air-dried and fixed before staining. Thick films are not fixed, which allows the RBCs to be lysed and concentrates the parasites. Thick films can be helpful in detecting small numbers of parasites, but species identification is best in thin films. For good stain definition, blood films should be stained as soon as possible. *Babesia* are found within RBCs, and all divisional stages - ring (annular) stages, pear-shaped (pyriform) trophozoites either singly or in pairs; and filamentous or amorphous shapes – may be detected simultaneously. However, filamentous or amorphous forms are usually seen in animals with very high levels of parasitemia. *B. bovis* trophozoites are small (usually 1-2 µm x 0.5-1µm) and often paired, and are usually centrally located in the RBC. *B. divergens* resembles *B. bovis*, but the pairs are often found at the edge of bovine RBCs. *B. bigemina* is much larger (2.5-3.5µm x 1–1.5 µm). Pairs of *B. bovis* and *B. divergens* trophozoites usually occur at obtuse angles to each other, while those of *B. bigemina* tend to appear at an acute angle or almost parallel. Morphological variability may make precise species identification difficult, and other species can resemble the major cattle parasites. For instance, *B. ovata* closely resembles *B. bigemina*.

PCR tests can be used to diagnose clinical cases and distinguish species of *Babesia*. Other genetic tests, including loop mediated isothermal amplification (LAMP) assays and a PCR-ELISA have been published for some organisms. Genetic tests are particularly useful in carriers. However, they may amplify some closely-related *Babesia*, and even sequencing of commonly amplified segments is not always sufficient to distinguish some wildlife *Babesia* from *B. divergens* unless detailed genetic analyses are conducted. *In vitro* culture of *Babesia* or animal inoculation are not employed routinely for diagnosis; however, these techniques are very sensitive and could be useful in some situations. Calves can be used to isolate cattle *Babesia*, but Mongolian gerbils can also be employed for *B. divergens*. Both *in vitro* culture and animal inoculation can take weeks. Animal inoculation is generally discouraged if alternative methods are available.

Serology is mainly employed in surveillance and export certification. Antibodies to *Babesia* can be detected with ELISAs or indirect fluorescent antibody (IFA) tests. ELISAs for *B. bovis*, *B. bigemina* and *B. divergens* have replaced IFA in many countries. ELISAs for other species of cattle *Babesia* have also been published. Immunochromatographic tests have been developed for some organisms, including *B. bovis* and *B. bigemina*, and immunoblotting has been described. Cross-reactivity with less pathogenic species of *Babesia* and some other piroplasms may complicate the interpretation of serological tests.

Treatment

In endemic areas, sick animals should be treated as soon as possible with an antiparasitic drug. Imidocarb is used most often. Where it is available, diminazene aceturate can also be used in cattle infected with *B. bigemina* or *B. ovata*. However, it is reported to be less effective against *B. bovis* and *B. divergens*. Treatment is most likely to be successful if the disease is diagnosed early, and may fail in very sick animals. Blood transfusions and other supportive therapy may also be necessary, but are typically used only in valuable cattle.

Control

Disease reporting

Veterinarians who encounter or suspect bovine babesiosis should follow their national and/or local guidelines for disease reporting. In the U.S., infections with *B. divergens*, *B. bovis* and *B. bigemina* should be reported immediately to state or federal authorities.

Prevention

Cattle are vaccinated against babesiosis in some countries, using live attenuated *B. bovis*, *B. bigemina* and/or *B. divergens.* Typically these vaccines are administered to 3-9 month-old cattle, which are naturally resistant to illness. Older animals should be monitored after vaccination and treated if clinical signs develop. However, the current vaccines produced in some countries are reported to be fairly safe even in this age group. Animals may also administered chemoprophylaxis with imidocarb, which protects them from clinical signs while they develop immunity. Some farms may choose to stock *Bos indicus* cattle, which are more resistant to the effects of babesiosis than *Bos taurus*. Tick control can reduce animals' exposure to *Babesia*, but stringent control may affect the boosting provided by repeated exposures, potentially increasing their susceptibility. The development of resistance to acaricides is also a concern. A vaccine against cattle ticks is available in South America, but a vaccine formerly used in Australia is no longer marketed. Environmental modification of tick habitats may reduce the number of ticks, but such changes may be difficult and/ or ecologically undesirable, and ticks sometimes persist in certain microenvironments. Natural endemic stability (see Morbidity and Mortality) is unreliable as the sole control strategy, as it can be affected by climate, host factors and management.

Eliminating babesiosis is difficult once an organism has been introduced into a region where there are

competent tick vectors; however, stamping out or other measures may be successful in some cases. *B. bovis* and *B. bigemina* were eradicated from the U.S. in an intensive campaign that included periodic acaricide treatment of all cattle. However, such extensive programs are rarely feasible: the U.S. program took 40 years in most of the country and longer in Florida, where cattle ticks persisted on deer. Since eradication, the disease-free status of the U.S. has been maintained by a quarantine buffer zone along the southern border, together with treatment of imported cattle for ticks. Threats to this program include the increasing numbers of cattle ticks with multiple acaricide resistance in Mexico, illegal movement of cattle, and the apparent persistence of host ticks in some locations in the quarantine buffer zone. In recent years, cattle ticks have been found occasionally on cattle or wild deer outside the quarantine buffer zone.

Disinfectants and sanitation are ineffective in preventing the transmission of babesiosis, but care should be taken not to transfer blood from infected animals.

Morbidity and Mortality

The percentage of tick larvae infected with cattle *Babesia* is influenced by the level of parasitemia in the host; however, some sources indicate the overall prevalence of *B. bovis*, *B. bigemina* and *B. divergens* in tick populations is low. Infections with *B. bigemina* are usually, though not always, more common than *B. bovis* in cattle. Some European countries have recently reported that bovine babesiosis due to *B. divergens* has decreased, for reasons that are still unclear.

Calves up to 9-12 months of age are relatively resistant to clinical signs, with some reports suggesting a lesser degree of resistance until they are around 2-2.5 years. In areas where the host ticks are active year-round, cattle tend to become infected when they are young, and are subsequently immune. This endemic stability can be upset, resulting in outbreaks, if changes in the weather, acaricide treatment or other factors decrease tick numbers and animals do not become infected during the critical early period. Outbreaks also occur in temperate regions where cold seasons interrupt tick-borne transmission for a time, and when susceptible animals are introduced to endemic regions or infected ticks enter new areas. Factors that may influence the incidence and severity of bovine babesiosis include the species and strain of the parasite, host factors such as stress or poor nutrition, and the species of cattle. *Bos indicus* and their crosses are more resistant to clinical signs than *Bos taurus*, although this resistance is not absolute. The overall mortality rate for bovine babesiosis is reported to be 5-10%, even with treatment. *B. bovis* usually causes more severe illnesses than *B. bigemina or B. divergens*, and mortality can reach 50-100% in untreated animals infected with this organism. The prognosis is guarded once hemoglobinuria develops, and CNS signs suggest a poor prognosis.

Infections in Humans

Incubation Period

Clinical signs are reported to occur within 1-3 weeks of exposure.

Clinical Signs

In people who have had splenectomies, clinical cases caused by *B. divergens* are usually characterized by the acute onset of severe hemolytic anemia. Some patients report weakness and discomfort before the overt signs develop. Most cases progress rapidly and can quickly become life-threatening. Common symptoms include hemoglobinuria, jaundice, persistent high fever, chills and sweats, headache, myalgia, and lumbar and abdominal pain. Vomiting and diarrhea may also be seen. Complications of renal failure, pulmonary edema and respiratory distress are frequently reported. Ecchymoses, petechiae, congestive heart failure, hepatic complications, multi-organ failure, shock and coma may also be seen. Convalescence can be prolonged in survivors.

Diagnostic Tests

Babesiosis in humans is usually diagnosed by PCR and the direct observation of parasites in stained blood smears. The paired piriform trophozoites of *B. divergens* tend to occur in the center of human RBCs, rather than at the periphery as in cattle. In most cases, parasitemia is high in splenectomized patients infected with this organism. Automated blood analyzers can miss *Babesia*.

Clinical cases caused by *B. divergens* usually progress quickly, and patients do not normally have detectable antibody titers at the time of the illness. However, serology can be employed retrospectively to help confirm the diagnosis. The availability of serological tests can be limited at diagnostic laboratories, as this illness is uncommon. IFA, ELISAs, immunoblotting and other assays have been described, but very few human cases have been reported, and the sensitivity and specificity of these tests is still unclear. Antibodies to *B. divergens* can cross-react with other zoonotic members of the *Babesia divergens*/ *B. odocoilei* complex. There may also be cross-reactivity with organisms such as *Plasmodium* spp. or *Toxoplasma gondii*, and false positive reactions caused by autoimmune diseases.

Treatment

B. divergens is treated with antiparasitic drugs, but supportive treatment, including blood exchange transfusion or red cell exchange apheresis, is also generally required due to the severity of the illness. The optimal drug treatment for this organism in humans is still unclear. Currently, the choice of drugs is usually based on recommended treatments for severe clinical cases caused by other *Babesia*.

Prevention

Prevention depends on avoiding tick bites by means such as protective clothing (e.g., long pants with the cuffs tucked into socks) and tick repellents. Skin and clothing should be inspected for ticks after potential exposure, and any attached ticks should be promptly removed. Environmental modifications such as keeping the grass mowed and removing leaf litter might help reduce tick numbers around the home, if a high-risk individual lives where *I. ricinus* is common.

Morbidity and Mortality

Although some species of *Babesia* can affect healthy people, all of the clinical cases caused by *B. divergens* seem to have occurred in people who had splenectomies. Fewer than 50 clinical cases have been reported in Europe since the 1950s. Most were life-threatening and progressed very rapidly. The majority of cases were fatal in the past; however, the case fatality rate has decreased to approximately 40% with modern antiparasitic drugs and supportive therapy.

While *Babesia* found in other animals (e.g., *B. microti* of rodents) can cause serious illnesses in people who are immunocompromised but have an intact spleen, there are currently no reports that *B. divergens* affects these individuals. One recent clinical case occurred in a person infected with HIV-1; however, he also had a splenectomy. Whether immunocompetent individuals can be infected with *B. divergens* is also unclear. Several surveys found antibodies to *Babesia* in approximately 1-2% of asymptomatic blood donors in European countries, none of whom recalled an illness consistent with babesiosis. Slightly higher rates (6%) were reported in people evaluated for anti-*Babesia* antibodies in a Lyme disease screening program, including people who were asymptomatic but had recently been exposed to ticks. Whether such antibodies are due to *B. divergen*s or other species of *Babesia* remains to be determined.

Internet Resources

Australian and New Zealand Standard Diagnostic Procedure for Tick Fever
http://www.agriculture.gov.au/SiteCollectionDocuments/animal/ahl/ANZSDP-Tick_borne_diseases.pdf

Queensland Government, Australia. Tick Fever Diagnosis Advice and Laboratory Services (includes link for making smears for diagnosis)
http://www.dpi.qld.gov.au/cps/rde/dpi/hs.xsl/4790_6241_ENA_HTML.htm

Queensland Government, Australia. Making smears for tick fever diagnosis
http://www.dpi.qld.gov.au/cps/rde/dpi/hs.xsl/4790_6224_ENA_HTML

The Merck Veterinary Manual
http://www.merckvetmanual.com/

United States Animal Health Association. Foreign Animal Diseases
http://www.aphis.usda.gov/emergency_response/downloads/nahems/fad.pdf

World Organization for Animal Health (OIE)
http://www.oie.int

OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals
http://www.oie.int/international-standard-setting/terrestrial-manual/access-online/

OIE Terrestrial Animal Health Code
http://www.oie.int/international-standard-setting/terrestrial-code/access-online/

Acknowledgements

This factsheet was written by Anna Rovid Spickler, DVM, PhD, Veterinary Specialist from the Center for Food Security and Public Health. The U.S. Department of Agriculture Animal and Plant Health Inspection Service (USDA APHIS) provided funding for this factsheet through a series of cooperative agreements related to the development of resources for initial accreditation training.

The following format can be used to cite this factsheet. Spickler, Anna Rovid. 2018. *Bovine Babesiosis*. Retrieved from http://www.cfsph.iastate.edu/DiseaseInfo/factsheets.php.

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*Link is defunct