

# BOVINE BABESIOSIS

Aetiology Epidemiology Diagnosis Prevention and Control References

## AETIOLOGY

### **Classification of the causative agent**

Bovine babesiosis (BB) is a tick-borne disease of cattle caused by the protozoan parasites of the genus *Babesia*, order Piroplasmida, phylum Apicomplexa. The principal species of *Babesia* that cause BB are: *Babesia bovis*, *Babesia bigemina* and *Babesia divergens*. Other *Babesia* that can infect cattle include *B. major*, *B. ovata*, *B. occultans* and *B. jakimovi*.

### **Resistance to physical and chemical action**

This agent does not survive outside its hosts and can only be transmitted through a tick vector. Therefore, parameters associated with resistance to physical and chemical actions (such as temperature, chemical/disinfectants, and environmental survival) are not meaningful. Susceptibility to medicines and vaccines are described under "Prevention and control".

## EPIDEMIOLOGY

All *Babesia* are transmitted by ticks with a limited host range. The principal vectors of *B. bovis* and *B. bigemina* are *Rhipicephalus* spp. ticks and these are widespread in tropical and subtropical countries. The major arthropod vector of *B. divergens* is *Ixodes ricinus*. BB is principally maintained by subclinically infected cattle that have recovered from disease. Morbidity and mortality vary greatly and are influenced by prevailing treatments employed in an area, previous exposure to a species/strain of parasite, and vaccination status. In endemic areas, cattle become infected at a young age and develop a long-term immunity. However, outbreaks can occur in these endemic areas if exposure to ticks by young animals is interrupted or immuno-naïve cattle are introduced. The introduction of *Babesia* infected ticks into previously tick-free areas may also lead to outbreaks of disease.

### **Hosts**

- *B. bovis* and *B. bigemina*
  - cattle
  - water buffalo (*Bubalus bubalis*) and African buffalo (*Syncerus caffer*)
  - reports of disease in white-tailed deer (*Odocoileus virginianus*) in Mexico
- *B. divergens*
  - cattle and reindeer (*Rangifer tarandus*)
  - Mongolian gerbils (*Meriones unguiculatus*); other peridomestic rodents are resistant to disease
  - Splenectomised humans and non-human primates are highly susceptible
  - Experimental infection with no clinical signs have been documented in splenectomised ungulates including mouflon (*Ovis musimon*), red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), and fallow deer (*Dama dama*)

### **Life Cycle and Transmission**

- BB is principally transmitted by means of ticks
  - Tick vectors of *Babesia bigemina*: *Rhipicephalus microplus* (formerly *Boophilus microplus*) and *Rhipicephalus annulatus* (formerly *Boophilus annulatus*); *Rhipicephalus decoloratus*, *Rhipicephalus geigy*, and *Rhipicephalus evertsi* are also competent vectors
    - *B. bigemina* transmitted by feeding of adult and nymphal stages of one-host *Rhipicephalus* spp. ticks
  - Tick vectors of *Babesia bovis*: *Rhipicephalus microplus* and *Rhipicephalus annulatus*; *Rhipicephalus geigy* is also a competent vector
    - *B. bovis* transmitted by feeding of larval stages of one-host *Rhipicephalus* spp. ticks

- Tick vectors of *Babesia divergens*: principal vector is *Ixodes ricinus*
  - *Ixodes ricinus* is a three-host tick with only adult stages feeding on vertebrates (eg. cattle)
- *Babesia* sporozoites are inoculated into the vertebrate host by ticks and invade red blood cells (RBCs) where they transform into trophozoites
  - These grow and divide into two round, oval or pear-shaped merozoites which, in turn, are capable of infecting new RBCs; the division process is then repeated
- *Babesia* parasites can be transmitted transovarially between tick generations; in the case of *Ixodes*, surviving up to 4 years without a vertebrate host
- *Babesia* may also be transmitted by fomites and mechanical vectors contaminated by infected blood
- Infrequently, calves can become infected *in utero*

### **Sources of infection**

- Blood infected with *Babesia* parasites and associated vectors of infected blood (especially ticks, but also by mechanical means)

### **Occurrence**

BB is found in areas where its arthropod vector is distributed, especially tropical and subtropical climates. *Babesia bovis* and *B. bigemina* are more widely distributed and of major importance in Africa, Asia, Australia, and Central and South America. *Babesia divergens* is economically important in some parts of Europe and possibly northern Africa.

**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**

Incubation period is often 2–3 weeks or longer after tick infestation. Shorter incubation periods have however been documented in the field and through experimental inoculation (4–5 days for *B. bigemina* and 10–12 days for *B. bovis*).

### **Clinical diagnosis**

Clinical manifestations of disease associated with BB are typical of a haemolytic anaemia disease process but vary according to agent (i.e. species of parasite) and host factors (i.e. age, immune status). BB is predominantly observed in adult cattle with *B. bovis* generally being more pathogenic than *B. bigemina* or *B. divergens*. Infected animals develop a life-long immunity against re-infection with the same species and some cross-protection is evident in *B. bigemina*-immune animals against subsequent *B. bovis* infections.

#### *Babesia bovis*

- High fever
- Ataxia and incoordination
- Anorexia
- Production of dark red or brown-colored urine
- Signs of general circulatory shock
- Sometimes nervous signs associated with sequestration of infected erythrocytes in cerebral capillaries
- In acute cases: maximum parasitaemia (percentage of infected erythrocytes) in circulating blood is often less than 1%

#### *Babesia bigemina*

- Fever
- Haemoglobinuria and anaemia

- Production of dark red or brown-colored urine
- Nervous signs minimal or non-existent as intravascular sequestration of infected erythrocytes does not occur
- Parasitaemia often exceeds 10% and may be as high as 30%

#### *Babesia divergens*

- Parasitaemia and clinical appearance are similar to *B. bigemina* infections

### **Lesions**

- Lesions observed are those most often associated with an intravascular haemolytic condition
- Pale or icteric mucous membranes; blood may appear thin and watery
- Subcutaneous tissues, abdominal fat and omentum may appear icteric
- Swollen liver with an orange-brown or paler coloration; enlarged gall bladder containing thick, granular bile
- Enlarged, dark, friable spleen
- Kidneys appear darker than normal with possible petechial haemorrhages
- Bladder may contain dark red or brown-colored urine
- Possible oedema of lungs
- Petechiae or ecchymoses on surface of heart and brain

### **Differential diagnosis**

- Anaplasmosis
- Trypanosomiasis
- Theileriosis
- Bacillary haemoglobinuria
- Leptospirosis
- Eperythrozoonosis
- Rapeseed poisoning
- Chronic copper poisoning

### **Laboratory diagnosis**

#### **Samples**

- Several thick and thin blood smears collected from superficial skin capillaries (e.g. tip of the ear or tip of the tail) of live animals during the acute phase of the disease (appearance of fever)
  - thin blood films should be air-dried, fixed in absolute methanol for 1 minute and stained in 10% Giemsa stain for 20–30 minutes
    - blood films should be stained as soon as possible after preparation to ensure proper stain definition
  - thick films are made by placing a small drop (approximately 50 µl) of blood on to a clean glass slide. The droplet is air-dried, heat-fixed at 80°C for 5 minutes, and stained (without fixing in methanol) in 10% Giemsa for 15 minutes
  - unstained blood smears should not be stored with or near formalin solutions as it may affect staining quality
- If it is not possible to make fresh smears from capillary blood, sterile jugular blood should be collected into an anticoagulant such as ethylene diamine tetra-acetic acid (EDTA) (e.g. 1 mg/ml); heparin is not recommended as it may affect staining
  - The sample should be kept cool, preferably at 5°C, until delivery to the laboratory, blood films should be made and stained preferably within 2–3 hours of collection
  - *B. bovis* is sequestered and found in higher numbers in capillary blood, *B. bigemina* and *B. divergens* parasites are uniformly distributed through the vasculature
- Samples from dead animals should consist of thin blood films, as well as smears from organs
- Organ smears acquired at necropsy (in order of preference): cerebral cortex, kidney, liver, spleen and bone marrow
  - organ smears are made by pressing a clean slide on to a freshly cut surface of the organ or by crushing a small sample of the tissue between two clean microscope slides drawn lengthwise to leave a film of tissue on each slide

- organ smear is then air-dried (assisted by gentle warming in humid climates), fixed for 5 minutes in absolute methanol, and stained for 20–30 minutes in 10% Giemsa
- especially suitable for the diagnosis of *B. bovis* infections but unreliable if sample taken 24 hours or longer after death has occurred
- *Babesia* parasites can often be detected in blood taken from veins, in the lower limb region, one or more days after death
- Serum samples should also be collected

## Procedures

### Identification of the agent

- Microscopic examination of blood – traditional method of identifying agent in infected animals by microscopic examination of Giemsa-stained thick and thin blood films
  - stained smears are examined under oil immersion using (as a minimum) a x8 eyepiece and a x60 objective lens
  - thick blood films are especially useful for the diagnosis of low level *B. bovis* infections, as are organ smears
  - morphology of *Babesia* described in various sources, including *OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*
  - sensitivity of test can detect parasitaemias as low as 1 parasite in 10<sup>6</sup> red blood cells in thick blood films
  - *Babesia* species differentiation is good in thin films but poor in the more sensitive thick films
  - adequate for detection of acute infections, but not for detection of carriers where parasitaemias are very low
  - parasite identification and differentiation improved by using a fluorescent dye, such as acridine orange instead of Giemsa
- Polymerase chain reaction (PCR) assays - very sensitive particularly in detecting *B. bovis* and *B. bigemina* in carrier cattle
  - a PCR-enzyme-linked immunosorbent assay (ELISA) is reported to be at least 1000 times more sensitive than thin blood smears for detection of *B. bovis*
  - a number of PCR techniques have been described that can detect and differentiate species of *Babesia* in carrier infections
  - current PCR assays generally do not lend themselves well to large-scale testing; unlikely to supplant serological tests as the method of choice for epidemiological studies
  - PCR assays are useful as confirmatory tests and in some cases for regulatory testing
- *In-vitro* culture methods
  - used to demonstrate presence of carrier infections of *Babesia* spp.; *B. bovis* has also been cloned in culture
  - minimum parasitaemia detectable by this method depends on the facilities available and the skills of the operator but could be as low as 10<sup>-10</sup>, making it a very sensitive method for the demonstration of infection
  - method is 100% specific
- Animal inoculation is not suitable for diagnostic purposes
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### Serological tests

- *Babesia bovis* enzyme-linked immunosorbent assay
  - ELISA for diagnosis of *B. bovis* infection uses a whole merozoite antigen; undergone extensive evaluation
  - Competitive ELISAs using recombinant merozoite surface and rhoptry associated antigens of *B. bovis* have recently been developed; not yet been widely validated
- *Babesia bigemina* enzyme-linked immunosorbent assay
  - a competitive ELISA developed and validated in Australia is the only ELISA in routine use. It has been included in the *OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*
  - no other well-validated ELISA available for *B. bigemina*; due in part to the fact that antibodies to *B. bigemina* typically have poor specificity
  - ELISAs have also been developed for *B. divergens* using antigen derived from culture, *Meriones* or cattle, but none has been validated internationally

- Indirect fluorescent antibody (IFA) test
  - widely used to detect antibodies to *Babesia* spp., but the *B. bigemina* test has poor specificity
  - cross-reactions with antibodies to *B. bovis* in the *B. bigemina* IFA test are a particular problem in areas where the two parasites coexist
  - disadvantages of low sample throughput and subjectivity
- Complement fixation
  - has been used to detect antibodies against *B. bovis* and *B. bigemina*
  - used to qualify animals for importation into some countries
- Other tests: dot ELISA, slide ELISA, and latex and card agglutination tests
  - tests show acceptable levels of sensitivity and specificity for *B. bovis* and, in the case of the dot ELISA, also for *B. bigemina*
  - however, none of these tests appears to have been adopted for routine diagnostic use in laboratories other than those in which the original development and validation took place
  - adaptability of these tests to routine diagnostic laboratories is therefore unknown

**For more detailed information regarding laboratory diagnostic methodologies , please refer to Chapter 2.4.2 Bovine babesiosis in the latest edition of the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* under the heading “Diagnostic Techniques” .**

## **PREVENTION AND CONTROL**

### **Sanitary prophylaxis**

- Eradication of BB has been accomplished by elimination of tick vector
  - in areas where eradication of tick is not feasible or desirable, ticks are controlled by repellents and acaricides
- Reducing exposure of cattle to ticks
  - repellents, acaricides and regular inspection; animals and premises
  - control and eradication of the tick vector
- Cattle develop a durable, long-lasting immunity after a single infection with *B. bovis*, *B. divergens* or *B. bigemina*, a feature that has been exploited in some countries to immunise cattle against babesiosis
- Endemic environments should be monitored carefully
  - introduction of immuno-naïve animals
  - introduction of new species or strains of disease agent
  - interruptions in exposure to ticks and disease due to changes in climate, host factors and management
- Special care in possible mechanical infection of horses with contaminated blood

### **Medical prophylaxis**

Vaccine for *Babesia*:

- Live vaccine: most live vaccines contain specially selected strains of *Babesia* (mainly *B. bovis* and *B. bigemina*) and are produced in calves or *in vitro* in government-supported production facilities as a service to the livestock industries
  - caution should be used in their employment as they may be virulent in adult animals, may be contaminated with other disease agents and could lead to hypersensitivity reactions; usually used in younger animals
  - an experimental *B. divergens* vaccine prepared from the blood of infected *Meriones* has also been used successfully
- Killed vaccine: prepared from blood of *B. divergens*-infected calves; little information available on level and duration of the conferred immunity
- Other vaccines:
  - parasite proteins have been characterised but no effective subunit vaccine is available commercially
  - experimental vaccines containing antigens produced *in vitro* have been developed but the level and duration of protection against heterologous challenge are unclear

For more detailed information regarding vaccines, please refer to Chapter 2.4.2 Bovine babesiosis in the latest edition of the *OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* under the heading “Requirements for Vaccines and Diagnostic Biologicals”.

#### Endemic areas

- Clinically affected animals treated with an antiparasitic drug (diminazene diaceturate, imidocarb, amicarbalide); efficacy depends on timely detection early in disease
  - *Babesia* parasites can be cleared from carrier animals; reduces clinical signs
  - Imidocarb has been reported to protect animals from disease but allow development of immunity; caution in regard to residues in milk and meat
- Consideration can be given to blood transfusions and other supportive therapy, if appropriate

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*.

### **REFERENCES AND OTHER INFORMATION**

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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](mailto:scientific.dept@oie.int)). Last updated October 2009.