Bovine Babesiosis

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DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

Clinical signs and pathology

In natural infections, incubation periods usually vary from 8 to 15 days. In acute manifestations, fever (>40°C) is usually present for several days before the onset of other clinical signs: inappetence, depression, weakness and reluctance to move. Haemoglobinuria is often present especially in B. bigemina infections (hence the common name "redwater"). Anaemia and icterus are especially obvious in more protracted cases. Diarrhoea is common and pregnant cows may abort. Cerebral babesiosis, which occasionally develops in B. bovis infections, is manifested by hyperaesthesia, nystagmus, circling, head pressing, aggression, convulsions and paralysis; these signs may or may not accompany other signs of acute babesiosis.

Necropsy of uncomplicated ("typical") cases of babesiosis is characterised by light red, watery blood and the mucous membranes and carcase are paler than normal (these changes are due to anaemia). In many cases this pallor may be masked by icterus (Figure 3). The spleen is invariably enlarged and has a pulpy consistency (severe congestion) (Figure 4). The liver is swollen, friable and yellowish-brown in colour (degeneration, bile stasis). The hepatic surface may have an evenly mottled appearance, with lighter coloured periacinar areas (fatty degeneration to necrosis). The gall-bladder is distended with viscous bile which often contains dark brown granules up to 1 mm in diameter (bile inspissation). The intestinal content is usually diminished (anorexia) and yellowish in colour (bile-stained). The kidneys are mildly to moderately swollen and dark reddish-brown in colour (haemoglobinuric nephrosis) (Figure 5) or yellowish-brown (cholaemic nephrosis). The lungs are often oedematous, with foam present in the bronchi and trachea (probably due to agonal left heart failure). The heart itself is usually flabby and pale (degeneration, anaemia) and agonal endocardial and epicardial petechiae and ecchymoses may be present. In cases which survive for longer, mild to moderate transudation into the body cavities (hydrothorax, hydropéricardium, ascites) may be observed. The urine is discoloured and may be deep yellow to yellow-brown (bilirubinuria) or a clear port-wine colour (haemoglobinuria). It must be emphasised that the above description of the macroscopic lesions applies not only to typical cases of babesiosis but to any disease in which significant erythrolysis occurs. It can thus be used as a model for most of the other haemolytic diseases.
Laboratory confirmation

Thin blood films made from capillary blood are preferred; thick blood films are more sensitive, but species differentiation is more difficult. Blood of the general circulation may contain up to 20 times fewer *B. bovis* than capillary blood. In *B. bigemina* infections, parasitized cells are evenly distributed throughout the blood circulation. *Babesia bovis* parasitaemias are often low (<0.1%), even at the peak of the reaction, while *B. bigemina* parasites are usually more numerous and therefore easy to detect.

*Babesia* spp. develop only in the erythrocytes. Merozoites (Figure 6) penetrate the cell membrane with the aid of the apical complex, and transform into trophozoites that undergo merogony to give rise to two new merozoites. Cells containing more than two parasites are rare. Parasitaemia can exceed 20% at the peak of the clinical reaction.
Babesia bovis is a “small” Babesia measuring up to 2 µm in diameter, while B. bigemina is larger and can extend to the full diameter of an erythrocyte. Both species show considerable morphological variation, however, making it difficult to distinguish one from the other. Large forms of B. bovis are quite common.

Single B. bovis organisms are round, oval or irregular in shape while paired forms are piriform or club-shaped (Figure 7). The angle between the paired organisms is often, but not invariably, obtuse (“bow-tie” appearance).

Single forms of B. bigemina are round, elongated or amoeboid in shape (Figure 8). Paired forms are typically piriform with an acute angle between them (Figure 5 & 8).

Figure 6: Electron micrograph of two Babesia bigemina merozoites in an erythrocyte

Figure 7: Blood smear showing Babesia bovis. Note round trophozoites (bottom right) and ‘bow-tie’ configuration of merozoites

Figure 8: Blood smear showing Babesia bigemina. Note large, round trophozoite (left) and acute angle between two large, pear-shaped merozoites (right)
Diagnosis can also be confirmed by examination of brain smears. If post mortem changes have resulted in parasites no longer resembling typical *Babesia* parasites, comparison of parasitaemias in brain smears and peripheral blood smears can indicate the *Babesia* species involved. *B. bigemina* parasitaemias in peripheral and brain smears will resemble one another, while *B. bovis* parasitaemia in brain capillaries will tend to be substantially higher than that in peripheral smears.

For histopathological examination, specimens of brain, spleen, liver and lung should be submitted.

The indirect fluorescent antibody (IFA) test is specific for *B. bovis*, but cross-reactions with antibodies to *B. bovis* in the *B. bigemina* IFA are a particular problem. Unfortunately, in the standard IFA test the degree of serological cross-reaction that occurs between the four *Babesia* spp. present in southern Africa is such that accurate differentiation is sometimes difficult. Internationally validated enzyme-linked immunosorbent assays (ELISAs) for the diagnosis of *B. bovis* infection have been developed. There is still no similarly validated ELISA for *B. bigemina*.

Sequestration of parasitized red blood cells in the peripheral circulation and evidence of vascular stasis are striking in acute infections of *Babesia bovis* (Figure 9). Accumulations of haemosiderin and phagocytosed red blood cells are common in cells of the reticuloendothelial system, especially in the spleen, liver and lymph nodes. Other lesions include:

- degeneration and necrosis of the epithelium of the convoluted tubules in the kidneys and an accumulation of hyaline or granular casts in the tubular lumens;
- centrilobular hydropic or fatty degeneration to extensive centrilobular and midzonal hepatic necrosis and bile stasis;
- marked congestion of the sinusoids of the spleen and a reduced ratio of white to red pulp with the germinal centres containing few cells;
- oedematous and congested sinuses in the lymph nodes and depletion of lymphocytes in the germinal centres;
- oedema of the lungs in some cases;
- marked distension of the capillaries of the brain by parasitized red blood cells (perivascular haemorrhages are uncommon) (Figures 10 & 11);
- haemorrhages in the myocardium and hyaline degeneration of some myocytes; and
- degeneration of skeletal muscle fibres in the hind limbs.
Figure 9: Erythrocytes parasitised by *Babesia bovis* adhering to each other

Figure 10: Brain capillaries packed with erythrocytes parasitised by *Babesia bovis*
In the kidneys, the capillaries are not packed as tightly with parasitized red blood cells as are those in the brain. Capillaries in the lungs are packed with red blood cells, but only a small proportion of the cells are parasitized.

In *Babesia bigemina* infections, histological changes are less pronounced than in *B. bovis* infections, and sequestration of infected red blood cells and vascular stasis are not features of the infection. Changes in the kidneys and liver are similar to those caused by *B. bovis*. Extensive necrosis of the red pulp of the spleen is common and large thrombi may be present.

Haemolytic anaemia, which is characteristically macrocytic and hypochromic, is a feature of *B. bovis* infections. Packed cell volumes (PCV) may fall to less than 0.10, total erythrocyte counts to less than 3.0 x 106/µℓ, and total haemoglobin to less than 50 g/l. Severe anaemia is particularly evident in protracted cases, while peracute cases may die with little evidence of anaemia. In cattle that survive, parasitaemia levels start decreasing ~5 days after the onset of patency and evidence of erythrocytic regeneration can be detected 2 to 4 days later: anisocytosis, polychromasia, punctate basophilia, macrocytosis and reticulocytosis. Leukocytic changes are variable, ranging from leukopenia to leukocytosis.

Changes in blood chemistry reflect the consequences of circulatory stasis and hypotension, including renal and liver damage, and muscle degeneration. Characteristically, the following occur:

- significant increases in blood urea nitrogen and plasma creatinine levels,
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- marked increases in unconjugated bilirubin levels, plasma creatine kinase and lactate dehydrogenase levels,
- the presence of haemoglobin in the serum to a level that may be as high as 5 g/L,
- increased plasma concentrations of fibrinogen and soluble fibrin,
- proteinuria during the acute phase of the infection of which 15 to 20 per cent is haemoglobin and 70 to 75 per cent albumin,
- metabolic and respiratory alkalosis as shown by elevated bicarbonate, excess base levels and a lowered pCO2, and
- a terminal increase in lactate and pyruvate levels.

Haemolytic anaemia, the outstanding feature in *Babesia bigemina* infection, is very similar to that seen in *B. bovis* infections. Rd blood cell destruction occurs more rapidly in severe cases however, and is accompanied by precipitous falls in PCVs, red blood cell counts and haemoglobin values. Osmotic fragility of the red blood cells increases during the acute phase of the infection, and serum haemoglobin levels are high in acute cases. Evidence of kidney and liver damage is similar to that seen in *B. bovis* infections.

**Differential diagnosis**

Babesiosis in bovines could be confused with anaplasmosis, but the latter generally leads to rumen stasis and constipation. Other causes of haematuria or haemoglobinuria may lead to a suspicion of babesiosis. Cerebral babesiosis could be confused with heartwater.