An account on equine babesioses *

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Summary: A review of the distribution of Babesia equi and Babesia caballi, the transmission of these protozoa by ticks and the immune response of infected horses, in addition to a brief survey of diagnostic tests and chemotherapy.

KEYWORDS: Babesia caballi – Babesia equi – Horse diseases – Protozoal infections – Reviews.

INTRODUCTION

Equine piroplasmosis or babesioses are tick-borne protozoal diseases of horses, mules, donkeys and zebras. The aetiological agents are blood parasites named Babesia equi and Babesia caballi. Infected animals may remain carriers of these parasites for long periods and act as sources of infection for tick vectors.

DISTRIBUTION

B. caballi and B. equi are frequently associated because they share common vectors. However, infections with B. equi are more prevalent than B. caballi infections. Both infections are widespread throughout tropical and subtropical zones, but B. caballi infections extend further north.

The African continent as a whole is highly endemic for B. equi. The seroepidemiological studies conducted by the authors have shown that in Zaire and in the Sudan virtually all horses are infected with B. equi. Similar results were reported for zebras in East Africa by Young and Purnell (42). While B. caballi infections are very common in the Sudan, such infections were not identified in Zaire. Thus, the geographical distribution of B. caballi in Africa south of the Sahara requires further investigation (39).

Equine babesioses are probably endemic throughout Asia, except in Siberia and Japan. High prevalences have been reported from the Near and Middle East and from India (12). Recent reports indicate that B. equi and B. caballi are common in China and in Korea. However, autochthonous infections obviously do not occur on the Japanese islands (37).

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In Europe, equine babesioses extend from Portugal and Spain, through France and Italy to the whole of the Balkan peninsula, to Hungary, Romania, and from there to most of the European countries of the Commonwealth of Independent States (CIS), such as Moldavia, Ukraine, the southern parts of Russia and the States of the Caucasus region. In the forest belt of central Russia, *B. caballi* infections extend as far north as the 58th degree of latitude. This is the distribution area of *Dermacentor reticulatus*, an important vector tick.

Equine babesioses are not endemic in Ireland, the United Kingdom (UK), the Netherlands, the Scandinavian countries or Germany. Belgium, Switzerland, Austria, the Czech Republic and Poland are probably marginal areas where autochthonous infections may occur. Most infections of horses examined in the UK and Germany have been traced back to Spain, France, Italy or to the CIS.

Australia is the only continent where equine *Babesia* have not become established, although *B. equi* was introduced with imported horses in 1976. There was some spread of the infection, probably by contamination of injection needles or other instruments by blood. These foci could be extinguished because suitable vector ticks were obviously absent.

In the New World, both equine *Babesia* species are highly endemic in Latin America, with the exception of the southern parts of Chile and Argentina. Almost all horses examined from Colombia and Brazil were seropositive for *B. equi*, and nearly as many for *B. caballi*. Horses in these countries are often heavily infested with ticks. Similar reports have been published for nearly all Latin American countries, ranging from Cuba to Argentina.

*B. caballi* and *B. equi* were not introduced into the United States of America (USA) until 1959. *B. caballi* infections became established in Florida and some adjacent states in which a vector tick, *D. nitens*, occurred. Although further spread of *B. caballi* was prevented by intensive control measures, attempts to eradicate the disease in Florida have not yet been successful.

**THE PARASITES**

During the life cycle of *Babesia*, sporozoites are inoculated into horses by vector ticks (10, 24).

*B. caballi* sporozoites exclusively invade erythrocytes where they transform into trophozoites (20). Each trophozoite grows and divides into two pear-shaped merozoites, 2-5 µm long by 1.3-3 µm in diameter. These large paired merozoites joined at the posterior ends are considered to be a diagnostic feature of *B. caballi* infection.

*B. equi* sporozoites initially invade lymphocytes, where they develop into macroschizonts and microschizonts. Microschizonts produce micromerozoites which finally enter erythrocytes (30). Micromerozoites are round or amoeboid and 2-3 µm long (20). They reproduce by simultaneous formation of a merozoite tetrad, a so-called 'Maltese cross'. This is a characteristic feature of *B. equi* (15).

The taxonomic position of *B. equi* appears to be uncertain at present (30). Comparison of the small subunit ribosomal ribonucleic acid (ss RNA) genes of various *Babesia*, *Theileria* and *Cytoszoon* parasites indicates that *B. equi* falls into a distinct group, different from both the *Babesia* and the *Theileria* genera (1).
TRANSMISSION OF THE PARASITES

*B. caballi* is transmitted by ten species of the genera *Dermacentor*, *Hyalomma* and *Rhipicephalus*, whereas *B. equi* is spread by eleven species of the genera *Dermacentor*, *Hyalomma*, *Rhipicephalus* and *Boophilus* (6, 10, 11, 26, 29, 34, 35, 36, 43). Ticks, rather than horses, are the reservoir of infection with *B. caballi* because the disease may persist in ticks throughout several generations, with trans-stadial and transovarial transmission (with the exception of *Rh. evertsi evertsi*). Infected horses are the reservoir of infection with *B. equi*, not vector ticks, because there is no transovarial but only stage-to-stage transmission.

*B. caballi* is transmitted in Europe by at least six tick species, five *Dermacentor* and one *Hyalomma* species.

In the New World, the only known vector for *B. caballi* is the one-host tick *Dermacentor (Anocentor) nitens*, the so-called tropical horse tick. However, *D. albipictus*, the winter tick, may be another vector, as shown experimentally. Altogether, thirteen tick species of the genera *Dermacentor*, *Hyalomma* and *Rhipicephalus* have been incriminated as vectors of *B. caballi*. This accounts for the high incidence and wide distribution of this parasite in the equine population. However, almost nothing is known of the transmission of *B. caballi* in the tropics and in the Southern Hemisphere. In Latin America, the highly abundant *D. nitens* is probably the principal vector.

*B. equi* is known to be transmitted in the Old World by at least eight tick species of the genera *Dermacentor*, *Hyalomma* and *Rhipicephalus*. No vector is known for *B. equi* in the New World, even though this parasite infects most of the horses in Latin America, as indicated above.

*B. equi* is usually acquired by nymphs and transmitted by male and female ticks. Single male ticks can transmit *B. equi* to several horses because of the longevity and mobility of such ticks. *Hyalomma* males, for instance, may infest horses or other hosts for several weeks or even months. This facilitates the spread of infection.

*B. equi* is highly infective for nymphs of *H. anatolicum* and *R. turanicus*: 85% to 92% of the nymphs may acquire infection despite low parasitaemias. Up to seventeen acini of the salivary glands may become infected in an ensuing adult tick. Many thousands of sporozoites are produced in a single acinus cell. Nymphs of the two tick species have become infected by feeding on a horse that was negative in the complement fixation (CF) test. These ticks transmitted *B. equi* to other horses in the adult stage. Hence, even CF-negative horses may constitute a source of infection for suitable vector ticks (10).

Besides the fact that the vector of *B. equi* is still unknown in Latin America, there may be other vector ticks not yet identified in the New World as well as in the Old World. *Babesia* may also adapt to development in additional tick species, as has happened before (e.g. *B. caballi* in *D. [Anocentor] nitens*).

*B. equi* can also be transmitted by blood-contaminated instruments. Intrauterine infection of the foetus can occur throughout the breeding life of the mare. Transmission of the parasites with semen has never been reported.

Parasitaemia rarely exceeds 1% in *B. caballi* infections. In *B. equi* infections, parasitaemia usually ranges from 1% to 7%, but may reach 80% in some cases. Horses usually remain carriers of *B. caballi* for only one to three years after infection, but may remain carriers for many years, perhaps for life, of *B. equi* (11).
CLINICAL SIGNS

The incubation period of *B. equi* infections after tick infestation varies from 12 to 19 days, and that of *B. caballi* from 10 to 30 days.

Babesioses can occur in acute, sub-acute and chronic forms.

Acute cases of *B. caballi* infections are characterised by fever that usually exceeds 40°C, dyspnoea, congestion of mucous membranes, oedemas and anaemia. Icterus, anaemia and haemoglobinuria are more severe with *B. equi*.

In sub-acute cases, clinical signs are similar. In addition, affected animals show loss of weight, and the fever is sometimes intermittent. The mucous membranes vary from pale pink to pink, or pale yellow to bright yellow. Constipation may occur and is frequently followed by diarrhoea. Mild oedematous swelling of the distal part of the limbs sometimes occurs.

Chronic cases usually show non-specific, rather variable clinical signs, such as mild inappetite, poor performance and a drop in body mass. The spleen is usually found to be enlarged on rectal examination.

The erythrocytes, platelet counts and haemoglobin concentration are reduced in both infections. Acute infections are characterised by neutropenia and lymphopenia (5).

A rare peracute form, where horses are found either dead or moribund, has been reported (21).

ANTIBODY RESPONSE

Antibody levels rise to an early peak and thereafter decline during the chronic stage of infection. Antibodies persist at a low level all the time horses remain carriers. These antibodies are first detected 7 to 11 days after inoculation of infected erythrocytes in ponies, and reach a peak 30 to 45 days after inoculation (39). In *B. equi* infections, antibodies are detected over a longer period than in *B. caballi* infections. Antibodies can persist some months beyond the carrier state.

Maternal antibodies persist in the foal for one to four months after birth. In exceptional cases, they may persist for longer.

After challenge by inoculation, antibodies increase sooner than after the primary inoculation of *B. caballi*; however, the level of antibodies cannot be correlated with the resistance against the parasite developed after the first inoculation (9).

DIAGNOSIS

During the clinical stage, the parasites can be demonstrated microscopically in stained blood smears (23, 32). However, even in the acute stage, the demonstration of *B. caballi* is often difficult as the parasitaemia is very low (less than 0.1%). It is extremely difficult to diagnose the organisms in carrier animals by means of microscopic examination of blood smears. Serological testing is therefore recommended in carrier animals as a preferred method of diagnosis.
Serological tests

Various serological techniques have been used in the diagnosis of babesiosis, such as complement fixation (CF), indirect fluorescent antibody test (IFAT) and enzyme-linked immunosorbent assay (ELISA). A detailed description of antigen production and test protocol has been prepared by the Standards Commission of the Office International des Epizooties (OIE).

Complement fixation

The CF test is the primary serological test used to diagnose piroplasmosis (38). This test was introduced as the official test by the United States Department of Agriculture (USDA), and has been accepted by other countries to control horses destined to be imported into countries where the disease does not occur, but where vectors may be present (4, 9, 40). The test specifically identifies carrier horses of *B. equi* or *B. caballi*, but does not detect all infected horses. Furthermore, this test cannot evaluate sera with anticomplement activity (14).

Indirect fluorescent antibody test

The IFAT has been successfully applied to the differential diagnosis of *B. equi* and *B. caballi* infections (7, 22, 39). The IFAT is more sensitive than the CF test in detecting carrier horses and in testing the results of treatment. The recognition of a strong positive reaction is relatively simple, but any differentiation between weak positive and negative reactions requires considerable experience in interpretation (5), and reading of results is time-consuming. For these reasons, this test is difficult to standardise. Furthermore, the antigen has a shelf life of only one year (39).

At present, because the CF test may not identify all infected animals, especially those that have been treated, and because of the anticomplementary reactions produced by some sera, the IFAT is recommended as a supplementary test (11).

Enzyme-linked immunosorbent assay

The ELISA has also been used to detect antibodies to both species of the parasite in experimentally infected horses (13, 25, 33, 41). It is apparent that cross-reactions between *B. equi* and *B. caballi* occur. The Western blot appears to be the most specific test available. This should be used as an additional test for species identification (2, 3).

Recent advances in this field have shown that a recombinant *B. equi* merozoite protein and a specific monoclonal antibody (MAb), which defines this merozoite surface protein epitope, can be used in a competitive inhibition ELISA (C-ELISA) (19). This C-ELISA overcomes the problem of antigen purity, since the specificity of the test depends only on the monoclonal antibody used. A 94% correlation was shown between the C-ELISA and the CF test in detecting antibodies to *B. equi*. Limited data at this stage would suggest that the C-ELISA is specific for *B. equi* (18).

The red cell stages of *B. equi* and *B. caballi* can now be cultivated in vitro (11, 16, 17), making it possible to produce antigens from cultures and also to test drugs in vitro.

Deoxyribonucleic acid probes

Recently, sensitive and specific deoxyribonucleic acid (DNA) probes have been isolated for both *B. equi* and *B. caballi* (27, 28). In this test, parasite DNA is extracted from a blood sample, spotted onto a nylon membrane and then examined with the
relevant radiolabelled DNA probe. These probes have been shown to detect parasites in some carrier animals, but need further refinement. Sensitivity must be increased, for instance by the development of amplification methods such as polymerase chain reaction (PCR). However, even the sensitivity of PCR may not be sufficient to detect latent parasitaemias (8).

**BIOLOGICAL TESTS**

These tests are considered to be research tools rather than diagnostic tests. They may be necessary for the isolation of strains, etc.

**Isotest**

Large quantities of whole blood (500 ml) or, preferably, washed red cells are transfused into a susceptible splenectomised horse. This animal is then kept under close observation for clinical signs of disease. Diagnosis is confirmed by the presence of parasites in the red cells. It is evident that this test is a cumbersome and expensive exercise.

**Xenotest**

In an additional technique, vector ticks are fed on a suspect animal and the organism can then either be identified in the ticks, or through the transmission of the organism by the vector ticks to another susceptible animal.

**Culture**

It has been shown recently that *B. caballi* and *B. equi* can be isolated from carrier horses by using improved media for *in vitro* culture (15, 16, 17).

**TREATMENT**

**Therapy**

A variety of drugs have been used for the treatment of equine babesioses (in acute form). *B. equi* is relatively resistant to therapy and repeated treatments may be required to control the disease. Only the most important and commonly used drugs will be referred to here (5).

Diminazene (Berenil®, Hoechst, Germany) is effective in the treatment of disease caused by *B. caballi*, but not by *B. equi*, when given intramuscularly at 11 mg/kg body weight on each of two consecutive days.

**Elimination of infection**

Imidocarb (Imizol®, Carbesia®, Mallinckrodt, United Kingdom) can eliminate *B. caballi* infections with an intramuscular dose of 2.2 mg/kg body weight, repeated 24 h later. Even four treatments, 72 h apart, at 4 mg/kg body weight or more are usually not successful in eliminating *B. equi* from infected horses. This dose is detrimental to the animal. A toxic dose ranging from 4 to 8 mg/kg body weight may reduce CF titres. Such horses may become CF-negative for a few weeks or months, but remain positive to the IFAT, also remaining infective for vector ticks.
As indicated, none of the drugs currently used is effective in eliminating *B. equi*. The dosages required usually approach toxic levels, thus causing undesirable side effects. Such doses may be fatal for horses.

Elimination of *B. caballi* infections in horses is rarely recommended in endemic areas, but may be indicated when horses are to be moved from an endemic country to one free of the disease where vector ticks may be present (5).

No vaccines against *B. equi* or *B. caballi* infections are available.

**EPIDEMIOLOGY**

There are few reports on severe outbreaks, although several have been reported from India (12, 31), as were some others during the Second World War. In general, acute disease is uncommon in endemic areas.

There have been a few outbreaks in the USA, Germany and Switzerland, without tick vectors being involved. In these disease-free areas, *B. equi* had been transmitted by the use of contaminated needles or instruments. A horse may remain infected with *B. equi* for many years, perhaps for life. Ticks can acquire the infection while feeding on infected horses which are negative to the CF test. Thus, infected horses are the reservoir of infection with *B. equi*, not vector ticks, because there is no transovarial but only stage-to-stage transmission and, perhaps, intra-stadial transmission by male ticks.

On the other hand, *B. caballi* infections are self-limiting, usually lasting one to three years. It is not known whether ticks can acquire a *B. caballi* infection from seronegative horses (using the CF test) or from latently infected animals. It appears that horses are infective for ticks only during the rather short period of a patent *B. caballi* infection. Thus, ticks, rather than horses, are the reservoir of infection with *B. caballi* because the infection may persist in ticks throughout several generations, with trans-stadial and transovarial transmission (11).

Spreading of infection from marginal to disease-free areas (e.g. to Germany or England) has not yet occurred, although potential vector ticks of the genus *Dermacentor* do exist in these countries.

Tick infestation rates are close to 100% in some Latin American countries. Two principal tick species infest horses in the New World: the tropical horse tick, *D. nitens*, and *Amblyomma cajennense*. However, these two species did not transmit *B. equi* in the laboratory (10).

In the Old World, tick infestation rates appear to be low in most countries. *Dermacentor* species are more common at the northern boundary of the distribution area and in marginal areas, whereas *Hyalomma* and *Rhipicephalus* ticks predominate in southern Europe, North Africa and the Near and Middle East, including India and the southern regions of the CIS.

There is a potential risk of *Dermacentor*, *Rhipicephalus* and *Hyalomma* species becoming established in the New World or in Australia, if these ticks are introduced with horses or other hosts.

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Résumé : Les auteurs étudient la répartition géographique de Babesia equi et de B. caballi, la transmission de ces protozoaires par les tiques ainsi que la réponse immune chez les équidés infectés. Ils donnent également un bref aperçu des épreuves de diagnostic et de la chimiothérapie de la maladie.


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Resumen: Los autores hacen una revisión de la distribución de Babesia equi y B. caballi, de la transmisión de estos protozoarios por garrapatas y de la respuesta inmune de los caballos infectados. Junto a ello, examinan someramente las pruebas de diagnóstico y la quimioterapia para esta enfermedad.


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REFERENCES


